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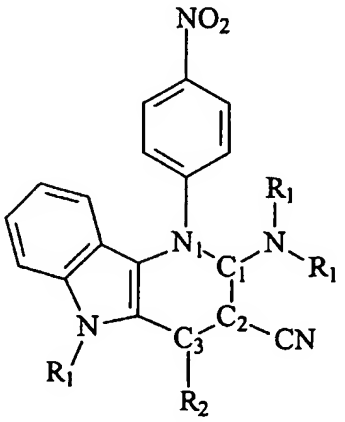
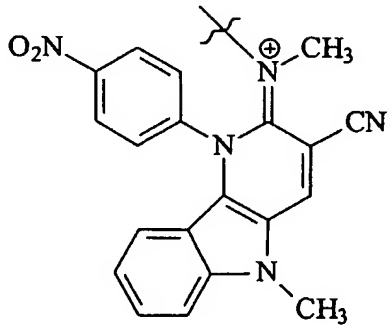
(54) Title: BENZOYLALKYLINDOLEPYRIDINIUM COMPOUNDS AND PHARMACEUTICAL COMPOSITIONS COMPRISING SUCH COMPOUNDS



(I)



(II)

(57) Abstract: The design, synthesis and antiviral activity of novel benzoylalkylindole-pyridiniums are disclosed. These compounds inhibit the reverse transcriptase enzymes of several retroviruses, including human immunodeficiency virus types 1 and 2, and simian immunodeficiency virus, and effectively inhibit viral replication thereby. Most of these compounds satisfy formula (1) where R_1 is independently selected from the group consisting of lower alkyl; N_1 is a nitrogen in a ring and is bonded to C_1 by either a single bond or a double bond; C_1 is a carbon atom in a ring, is bonded to N_1 by either a single bond or a double bond, and is bonded to C_2 by either a single bond or a double bond; C_2 is a carbon atom in a ring and is bonded to C_1 by either a single bond or a double bond, and is bonded by either a single bond or a double bond to C_3; and R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkyl amide, and formula (2). Compositions comprising effective amounts of such compounds are also described. These compounds and compositions can be used in a method for inhibiting the replication of retroviruses in a subject comprising administering an effective amount of the compound(s) or composition(s) comprising the compound, to a subject to inhibit retroviral replication.

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BENZOYLALKYLINDOLEPYRIDINIUM COMPOUNDS AND PHARMACEUTICAL COMPOSITIONS COMPRISING *SUCH COMPOUNDS*

REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 60/256,581, filed December 18, 2000.

FIELD

The present invention concerns benzoylalkylindolepyridinium compounds, pharmaceutical compositions comprising such compounds, and methods for making and using such compounds and compositions.

BACKGROUND

Viruses cause a variety of human and animal illnesses. Many are relatively harmless and self-limiting, but the other end of the spectrum includes acute life-threatening illnesses such as hemorrhagic fever, and prolonged serious illnesses such as hepatitis B and acquired immune deficiency syndrome (AIDS). Unlike bacterial infections, where numerous suitable antibiotic drugs are usually available, there are relatively few effective antiviral treatments.

A. Viruses

Viruses consist of a nucleic acid surrounded by one or more proteins. A virus's nucleic acid typically comprises relatively few genes, embodied either as DNA or RNA. DNA genomes may be single or double-stranded (examples include hepatitis B virus and herpes virus). RNA genomes may be single strand sense (so-called positive-strand genomes; examples include poliovirus), single strand or segmented antisense (so-called negative-strand genomes; examples include HIV and influenza virus), or double-stranded segmented RNA genomes (examples include rotavirus, an acute intestinal virus).

Retroviruses represent a particular family of negative stranded RNA virus. The term "retrovirus" means that in the host cell the viral RNA genome is transcribed into DNA. Thus, information is not passing in the "normal" direction, from DNA to RNA to proteins, but rather in a "retrograde" direction, from RNA to DNA. To accomplish this change in direction, a

retrovirus has one of a unique class of enzymes, referred to as reverse transcriptases. These enzymes are RNA-dependent DNA polymerases -- that is, they synthesize DNA strands using the viral RNA genome as a template. Each species of retrovirus has its own reverse transcriptase. Once the reverse transcriptase copies the retroviral RNA genome, it uses its inherent *DNA-dependent* DNA polymerase activity—that is, the ability to synthesize DNA copied from other DNA—to generate a double-stranded DNA version of the viral DNA genome.

HIVs (human immunodeficiency viruses) are retroviruses of the lentivirus subfamily. The two known subfamily members that infect humans are called HIV-1 and HIV-2 (simian immunodeficiency virus, or SIV, is a closely related lentivirus that infects monkeys). Once the virus gains entry into the body, it attaches to human immune cells that express the CD4 receptor on their surface (CD4+ cells). CD4+ cells (which include "helper" and lymphocytes and monocytes), become the primary repository for the virus. HIV-1 isolates are categorized into two broad groups, group M and group O. Group O comprises eight subtypes or clades, designated A through H.

B. Viral Therapeutics

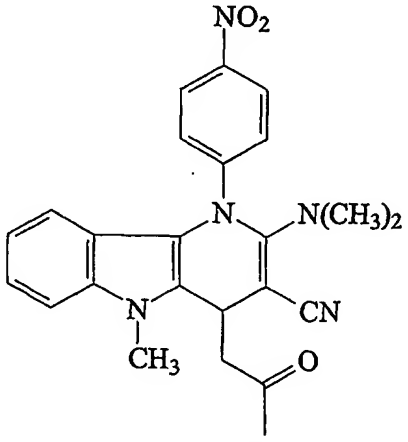
Currently, only a limited number of drugs are approved for treating viral infections, such as human immune deficiency virus Type 1 (HIV-1) infection. Two broad families of anti-HIV drugs include the viral protease inhibitors, and the reverse transcriptase (RT) inhibitors. There are three main classes of RT inhibitors: (1) dideoxynucleoside (ddN) analogs, (2) acyclic nucleoside phosphonate (ANP) analogs, and (3) non-nucleoside reverse transcriptase inhibitors (NNRTIs).

The ddN and ANP nucleoside analog drugs are phosphorylated inside the cell. Once phosphorylated, they bind to the RT's substrate binding site. This is the site where the RT binds nucleotides (dATP, dCTP, dGTP, or dTTP, collectively referred to as dNTPs) so that they can be added to the growing DNA chain. When a nucleoside analog drug binds to the RT substrate binding site, it is integrated into the DNA, just as a normal dNTP would. But the enzyme cannot subsequently add dNTPs onto the incorporated nucleoside analog. Thus, the two classes of nucleoside analogs function as "chain terminators," and thereby limit HIV replication. These drugs have proven clinically effective against HIV infection, but resistance rapidly emerges due to mutations in and around the RT active site.

NNRTIs do not require phosphorylation or function as chain terminators, and do not bind at the substrate (dNTP) binding site. Known NNRTIs bind to a specific region outside the RT active site, and cause conformational changes in the enzyme that render it inactive. Known NNRTIs are highly potent and relatively non-toxic agents that are extremely selective for inhibition of HIV-1 RT. However, like the nucleoside analogs, their use is limited by the rapid emergence of resistant strains. In addition, they do not inhibit the RT activity of HIV-2, SIV and possibly some HIV-1 Group O isolates, nor do they prevent these viruses from replicating.

C. Pyrido-Indole Compounds

Ryabova et al. describe certain pyrido-indole compounds in "2-Formyl-3-Aryl-aminoindoles in the Synthesis of 1,2- and 1,4-Dehydro-5H-Pyrido-[3,2-b]-Indole (δ carboline) Derivatives," *Pharmaceutical Chemistry Journal*, 30:579-583 (1996). For example, Ryabova et al. describe 1-(4-nitrophenyl)-2-dimethylamino-3-cyano-4-(2-oxo-propyl)-5-methyl-1,4-dehydro-5H-pyrido [3,2-b]-indole (Compound 2).



Compound 2

No biological data is provided for this compound.

D. Conclusion

The treatment of viral diseases, such as HIV disease, has been significantly advanced by the recognition that combining different drugs with specific activities against different biochemical functions of the virus can help reduce the rapid development of drug resistant

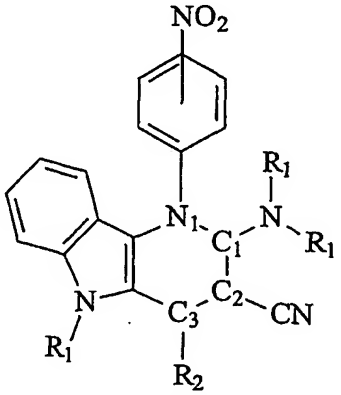
viruses. However, even with combined treatments, multi-drug resistant strains of the virus have emerged. Therefore, there is a continuing need to develop new drugs, particularly antiviral drugs that act specifically at different steps of the viral infection and replication cycle.

SUMMARY

The disclosed invention provides new compounds and pharmaceutical compositions comprising such compounds, particularly antiretroviral compounds and compositions that address many of the problems noted above. These compounds are referred to as benzoylalkylindolepyridinum compounds (BAIPs).

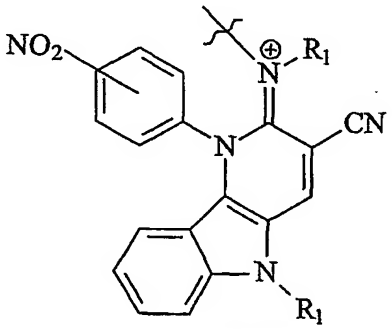
BAIPs of the disclosed invention are effective against viral infections, such as HIV isolates that have developed mutations rendering conventional drugs ineffective in their treatment. These BAIPs apparently do not require intracellular phosphorylation, nor bind to the RT active site, which distinguishes their mechanism of action from the ddN and ANP nucleoside analog drugs. The BAIPs' also may be distinguished from the NNRTIs, in part because the BAIPs bind to a different site on the RT enzyme. Moreover, unlike the NNRTIs, BAIPs of the present invention have been shown to be effective for limiting HIV-1, HIV-2, and SIV proliferation. Thus, BAIPs are broadly antiviral, non-nucleoside, reverse transcriptase inhibitors (BANNRTIs).

Disclosed BAIPs of the present invention typically have general Formula I below.



Formula I

With reference to Formula I, R_1 is independently selected from the group consisting of hydrogen and lower aliphatic groups, particularly lower alkyl. N_1 is a nitrogen in a ring and is bonded to C_1 by either a single bond or a double bond. C_1 is a carbon atom in a ring, is bonded to N_1 by either a single bond or a double bond, and is bonded to C_2 by either a single bond or a double bond. If C_1 and C_2 are bonded by a double bond, then R_1 is other than methyl, R_2 is other than $-OCH_2COCH_3$, and/or the nitrophenyl group is other than *p*-nitrophenyl. Formula I indicates that the nitro group of the nitrophenyl moiety can be in various ring positions, such as *ortho, meta* or *para*. Disclosed compounds having the nitro group in the *para* position currently are the most biologically active compounds. C_2 is a carbon atom in a ring and is bonded to C_1 by either a single bond or a double bond, and is bonded by either a single bond or a double bond to C_3. R_2 is selected from the group consisting of hydrogen, lower aliphatic, particularly lower alkyl, such as methyl, lower aliphatic amide, particularly lower alkyl amide, and Formula II below.



Formula II

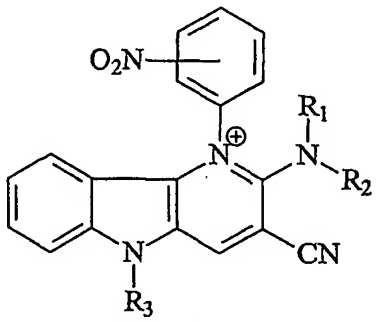
With reference to Formula II, R_1 is selected from the group consisting of hydrogen and lower aliphatic, particularly lower alkyl, such as methyl.

"Lower" as used herein refers to compound or substituents having 10 or fewer carbon atoms in a chain, and includes all position, geometric and stereoisomers of such substituents or compounds.

"Aliphatic" refers to compounds having carbon and hydrogen molecules arranged in straight or branched chains including, without limitation, alkanes, alkenes and alkynes.

"Alkyl" as used herein refers generally to a monovalent hydrocarbon group formed by removing one hydrogen from an alkane. An alkyl group is designated generally as an "R" group, and has the general formula $-C_nH_{2n+1}$.

Particular examples of disclosed compounds having Formula I also have Formula III.



Formula III

With reference to Formula III, R_1, R_2, and R_3 are independently selected from the group consisting of hydrogen and lower aliphatic groups, particularly lower alkyl groups, such as methyl. Compounds having Formula III can be made, amongst other methods, from compounds having Formula I. For example, such compounds can be made by treating a compound having Formula III with an acid. Working methods typically have used concentrated hydrochloric acid (HCl).

The present invention also provides compositions comprising amounts of a compound, or compounds, satisfying Formulas I and III above effective to treat diseases, particularly viral infections. Without being limited to one theory of action, one likely mechanism of action is inhibition of reverse transcriptase. Effective amounts therefore can be amounts sufficient to inhibit reverse transcriptase. Such compositions may further comprise inert carriers, excipients, diagnostics, direct compression binders, buffers, stabilizers, fillers, disintegrants, flavors, colors, lubricants, other active ingredients, other materials conventionally used in the formulation of pharmaceutical compositions, and mixtures thereof.

A method for treating a subject, particularly mammals, such as humans and simians, also is disclosed. The method first comprises providing a compound or compounds, or a composition comprising the compound or compounds, as described above. An amount of the compound(s) or composition(s) effective to inhibit viral replication is then administered to a subject. The effective amount typically should be as high as the subject can tolerate.

Administering the compound(s) or composition(s) can comprise administering topically, orally, intramuscularly, intranasally, subcutaneously, intraperitoneally, intralesionally, intravenously, or combinations thereof. The currently preferred administration method is intravenous.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph of various concentrations of Compound 2 (μM) versus percent control which illustrates the effects of Compound 2 on virus particles released from infected cells, where virus associated p24 antigen (◇) was quantitated by antigen capture assay, RT activity (□) was assessed by a homopolymeric(rA) template-primer system assay, and infectious units (△) were quantitated by titration of cell-free supernatant on MAGI cells.

FIG. 2 is a photograph of Western blot gels with AIDS patient serum or with polyclonal antiserum to HIV-1 RT protein.

FIG. 3 is graph of concentration of Compound 2 versus percent control showing decreased (1) RT activity levels (•), which were quantitated in the cell-free supernatant from TNF-α stimulated ACH2 cells in the presence of Compound 2, and (2) infectious units (□), which were quantitated in the cell-free supernatant from TNF-α stimulated ACH2 cells in the presence of Compound 2, (3) RT (○) of a separate sample, and (4) infectious units (❑) from a separate sample showing that under these conditions activities of RT and infectivity were recovered, where points on the graph represent means of triplicate tests from a representative experiment. The levels of RT activity were measured in virus harvested from drug-free TNF-α stimulated ACH2 cells after treatment of those preparations with either freshly prepared Compound 2 or with the fluid phase in which the virus had been cleared by centrifugation from the Compound 2 treated cultures.

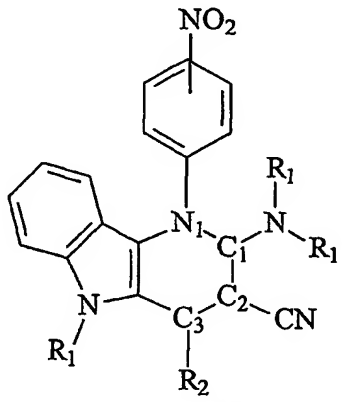
FIG. 4 is a graph of time-of-addition conditions illustrating inhibition of HIV-1 replication in cell-based, time-course assays comparing Compound 4 of the present invention to nevirapine and dextran sulfate added to the cultures at 0, 2, 4, 8 and 24 hours.

FIG. 5 is a is a graph of time-of-removal, time-course assays comparing Compound 4 of the present invention to nevirapine and dextran sulfate removed from the cultures at 2, 4, 8, 24 and 48 hours.

DETAILED DESCRIPTION

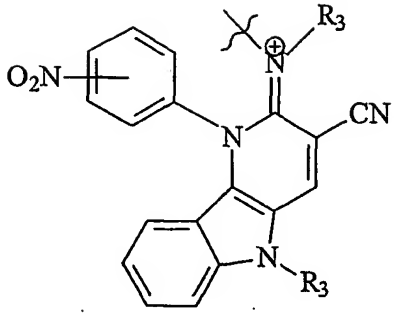
I. **Benzoylalkylindolepyridinium (BAIPs) Compounds**

BAIPs of the present invention typically satisfy general Formula 1 below:



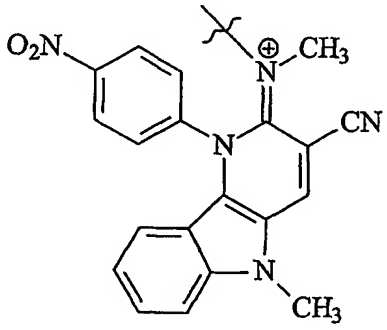
Formula I

With reference to Formula I, R_1 is independently selected from the group consisting of hydrogen and lower aliphatic, particularly lower alkyl, such as methyl ($-CH_3$). As used herein, "independently" means that each R_1 substituent may be different from or the same as the other R_1 substituents of Formula I. N_1 is a nitrogen in a ring, generally a six membered ring, and is bonded to C_1 by either a single bond or a double bond. If N_1 is bonded to C_1 by a double bond, then the nitrogen carries a positive charge, and such compounds may be administered as salts. C_1 is a carbon atom in a ring, generally a six membered ring, and is bonded to N_1 as previously described. C_1 also is bonded to C_2 by either a single bond or a double bond. If N_1 and C_1 are bonded together by a single bond, then C_1 is bonded to C_2 by a double bond. If N_1 and C_1 are bonded together by a double bond, then C_1 and C_2 are bonded together by a single bond. C_2 is a carbon atom in a ring, generally a six membered ring, and is bonded to C_1 as previously described. C_2 also is bonded by either a single bond or a double bond to C_3. If C_1 and C_2 are bonded together by a single bond, then C_2 and C_3 are bonded together by a double bond. If C_1 and C_2 are bonded together by a double bond, then C_2 and C_3 are bonded together by a single bond. R_2 is selected from the group consisting of hydrogen, lower aliphatic, particularly lower alkyl, such as methyl, lower aliphatic amide, particularly lower alkyl amide, and compounds having Formula II.

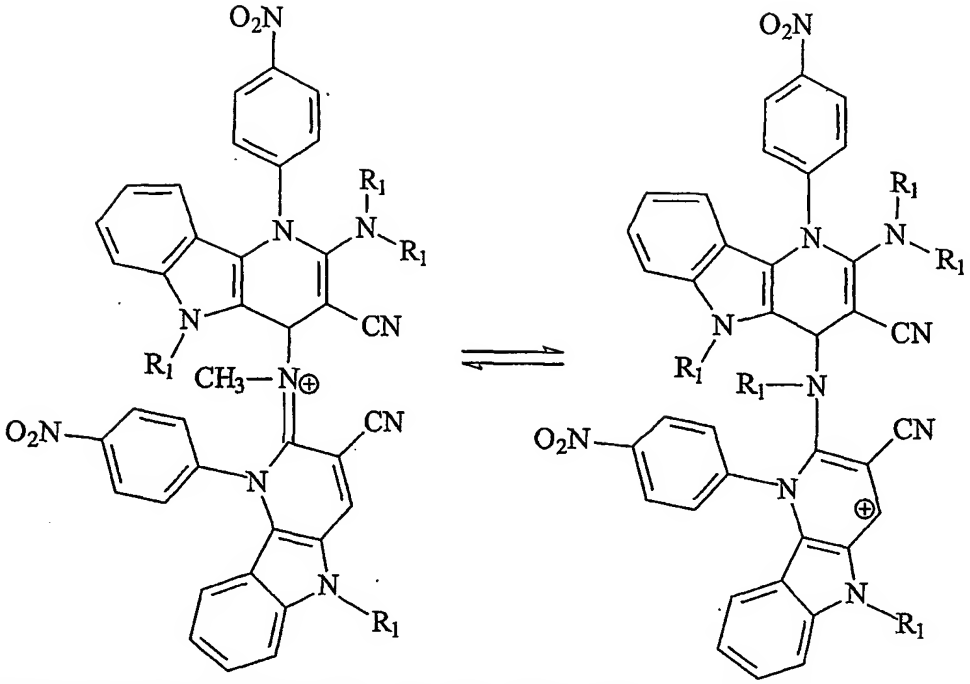


Formula II

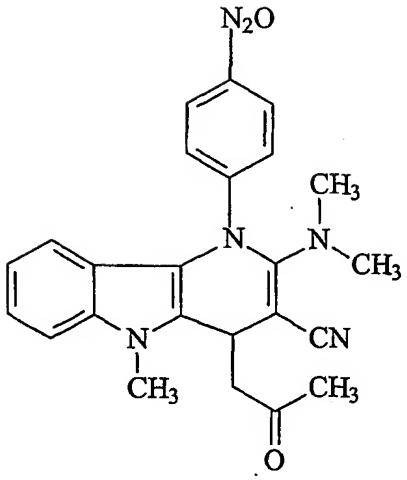
With reference to Formula II, R_3 is independently selected from the group consisting of hydrogen and lower aliphatic, particularly lower alkyl, such as methyl ($-CH_3$). An example of a compound according to the present invention which satisfied Formula II had the structure shown below.



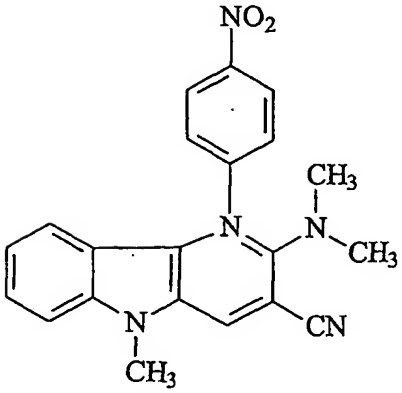
Compounds having R_2 = Formula 2 are believed to exist in two different forms, as shown below.



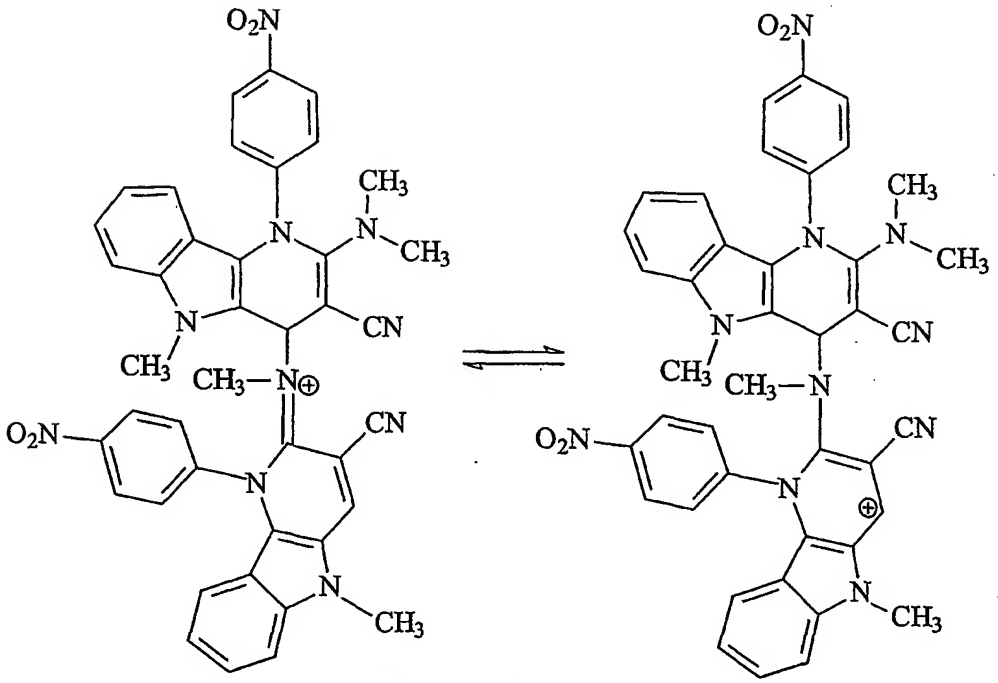
Examples of compounds that have been made and which satisfy general Formula I include Compounds 2, 4 and dimer 6.



Compound 2



Compound 4



Dimer Compound 6

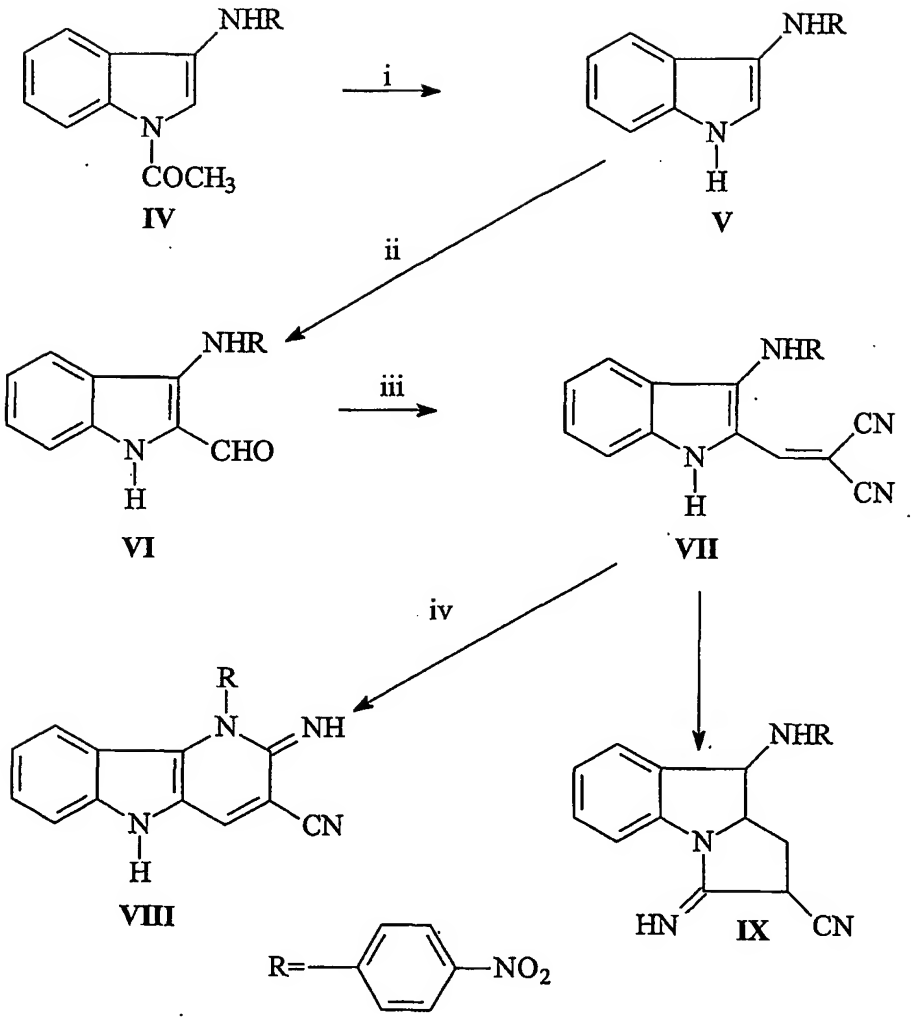
II. General Methods for Making BAIPs

Compound 2 can be made as described by Ryabova in "2-Formyl-3-Aryl-aminoindolesin the Synthesis of 1,2- and 1,4-Dehydro-5H-Pyrido-[3,2-b]-Indole (δ carboline) Derivatives," *Pharmaceutical Chemistry Journal*, 30:579-583 (1996), which is incorporated herein by reference. Other methods also can be used to make such compound, as well as other compounds according to the present invention.

With reference to Scheme 1, in a first such method IV was deacylated by action of Et_3N in methanol to form 3-p-nitrophenylaminoindole V, yield 80%, m.p. 220-222°C (MeOH), IR ν/cm^{-1}: 3350, 1590; MS *m/z* 253 (M^+). Formylation of V by treatment with Vilsmeier reagent produced the 2-formyl derivative VI, yield 96%, m.p. 237-238 °C (DMF-H_2O, 2:1); IR ν/cm^{-1}: 3290, 1640, 1600, 1575; ^1H NMR ([$^2H_6$]DMSO), δ: 9.88 (1H, s, CHO), 11.85, 9.41 (2H, 2s, NH, $NHC_6H_4NO_2$), 7.48 (4H, A_2B_2 system, $C_6H_4NO_2$), 6.95-7.59 (4H, m, arom. protons); MS *m/z* 281 (M^+).

Condensation of aldehyde VI with the dinitrile of malonic acid (both in the presence of Et_3N at 20°C or without Et_3N but under reflux) leads to dinitrile VII, yield 80% and 71%, respectively; a m.p. >270°C (dioxane); IR, v/cm^{-1}: 3390, 3290, 2210, 1570; ^1H NMR ($[^2H_6]$DMSO), 5: 8.19 (1H, s, CH), 11.17, 9.68 (2H, 2s, NH, $NHC_6H_4NO_2$), 7.52 (4H, A_2B_2 system, $C_6H_4NO_2$), 7.11, 7.67 (4H, m, arom. protons); MS *m/z* 329 (M^+). Cyclization of dinitrile VII can occur in either of two directions: with participation of *endo* (indole) or *exo* (at position 3) cyclic NH groups.

Heating VII in DMF-MeOH (1:1) caused intramolecular cyclization to form VIII isolated as the semihydrate (Scheme 1) yield 60%, m.p. 280°C (decomp., DMF-MeOH, 1 :1).



Scheme 1

Reagents and conditions for Scheme 1:

i. Et_3N, MeOH, reflux two hours.

ii. $POCL_3$-DMF, 5-10°C, 0.25 hours; addition of a solution of 3 in DMF, standing of the mixture (20°C, 18 hours)

iii. PrOH, $CH_2(CN)_2$, reflux 5 hours, or PrOH, PrOH, $CH_2(CN)_2$, Et_3N, 5 hours, 20°C.

iv. DMF-MeOH, 1: 1, reflux 0.25 hours.

Spectroscopic data for VII

IR ν/cm^{-1}: 3320, 2200, 1620, 1600, 1580.

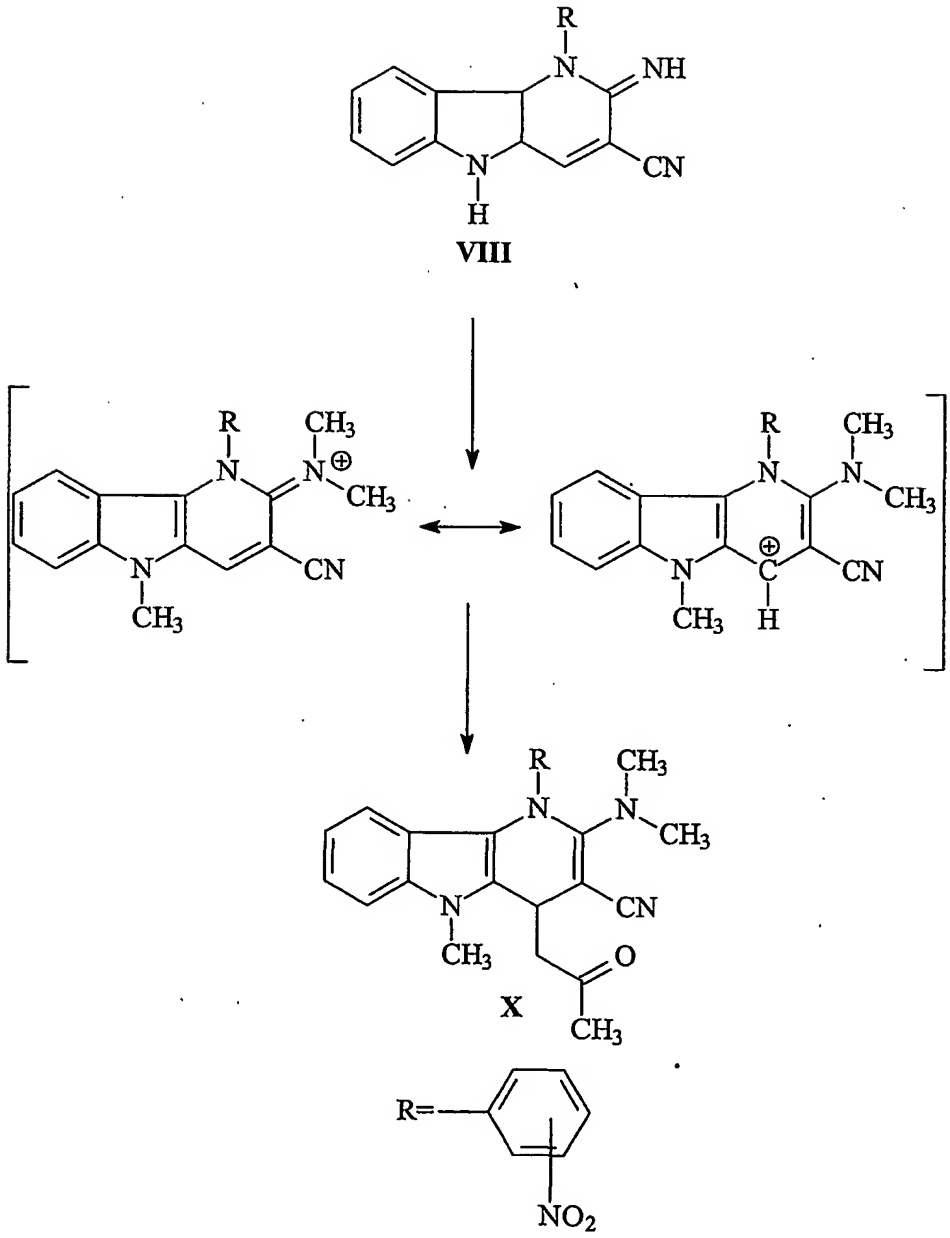
^{1}H NMR-DMSO-d_6, S: 6.17(bs, 2H), 5.91 (d, 1H, H-C^9), 6.74 (t, 1H, H-C^8)

^{13}C NMR ([$^2H_6$]DMSO) δ: 154.9 (C_2), 99.8 (C_3), 133.9 (C_4), 119.8 (C_{4a}), 114.5 (C_{9b}), 139.9 (C_{5a}), 128.8 (C_{9a}), 113.1, 119.9, 126.2, 127.1 (C_{6-9}), 119.9, 131.1 ($C_{2,3,5,6}$), 148.1, 144.1 ($C_{1,4}$), 117.7 (CN).

MS *m/z* 329 (M^+).

The structure of X as a 1,2-dihydro-δ-carboline derivative (and not the pyrrolo[l,2-*a*]indole derivative, VIII) was established by ^{1}H NMR and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum of IX ([$^2H_6$]DMSO) displays signals: 6.14 (1H, br.s, NH-imine), 5.91 (1H, d, *J* 9 Hz, H-C_9), 6.74 (1H, t), 7.23 (1H, t) and 7.42 (1H, d, *J* 9 Hz) (8,7,6 benzene ring protons), 8.20 (4H, A_2B_2 system, $C_6H_4NO_2$), 8.25 (1H, s, H-C4). The signal due to indole NH is absent. The characteristic peculiarity of the spectrum of this compound is the significant shift of the 9-H signal to lower field (5.91 as compared with 7.27-7.94 for aromatic protons in VIII). Such a displacement is caused exclusively by the screening influence of the anisotropy of the ring currents of the l-*p*-nitrophenyl substituent turned from the plane of the tricycle molecule (from Dreiding molecular models).

Scheme 2 shows an interesting and unexpected result obtained when VIII is methylated. Reacting VIII with methyl iodide in acetone in the presence of anhydrous K_2CO_3 adds the acetonyl anion to the molecule's 4 position, together with tris-alkylation. As a result, 1-nitrophenyl-2-dimethylamino-3-cyano-4-acetonyl-5-methyl-1,4-dihydropyrido[3,2-*b*]indole X is obtained, yield 75%, m.p. 198-199°C (MeOH-dioxane, 3:1). Scheme 3 is likely for the formation of X, which is the prodrug Compound 2.



Scheme 2

Reagent and conditions: MeI, acetone, anhydrous K_2CO_3, reflux 56-60 hours, MeI added to the reaction mixture every 7-8 hours.

Spectroscopic data for X

IR ν/cm^{-1}: 1720, 2190. 1H NMR ([$^2H_6$]DMSO) d: 3.75 (3H, s, NMe-indole), 2.90 (6H, br.s, NMe_2), 2.10 (3H, s, CH_2COMe) 2.69 (2H, AB system J_{hem}.17 Hz, J^1_{vic} 9 Hz, J^2_{vic} 5 Hz, CH_2COMe), 4.21 (1H, q, H-C_4), 7.89 (4H, A_2B_2 system, $C_6H_4NO_2$), 7.08-7.53 (4H, m, arom. protons). MS *m/z* 429 (M^+), 372 (M^+-CH_2COMe).

In Scheme 2, first tris-methylation appears to occur with formation of a positively charged species, and the acetonyl anion (formed in the reaction mixture in the presence of K_2CO_3) reacts at the electron-deficient position 4 to yield X. In the ^1H NMR spectrum of X (as distinct from VIII) a lower-field shift of the 9-H signal is not observed. It is known that the 1,4-dihydropyridine ring is not a flat system, and some data show that this ring has a boat conformation. Construction of molecular models for X, taking into account these data, shows that in this instance the p-nitrophenyl ring cannot influence the shape due to the above-mentioned anisotropic effect (as for VIII) and so the signals for all the protons in the condensed benzene ring are within the same range (7.08-7.53).

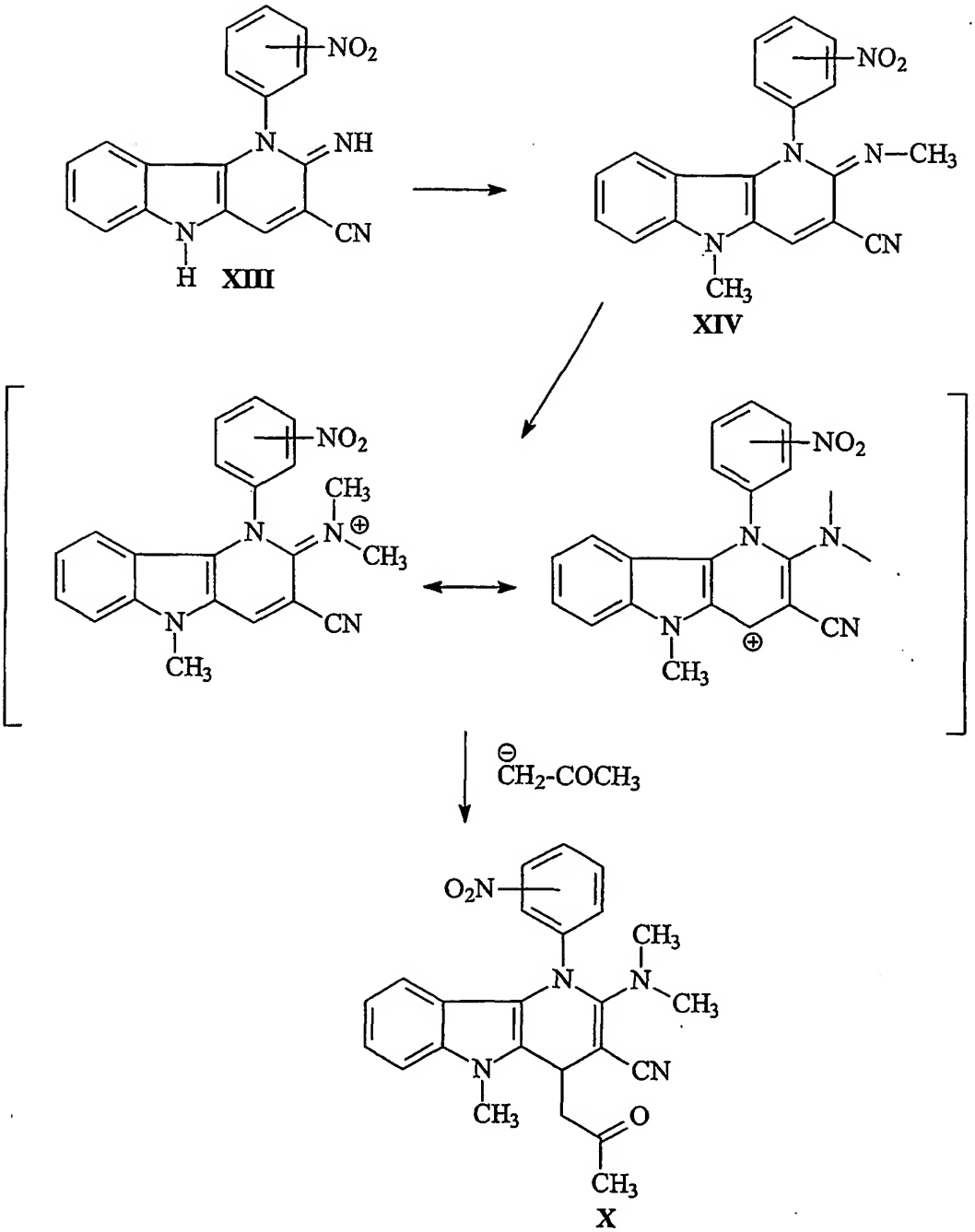
Ryabova et al., Khim.-Farm. Zh., 30: 42-45, (1996) reported the synthesis of 2-formyl-3-arylaminoindole derivatives by formylation of the corresponding 3-arylaminoindoles according to the Vilsmeier reaction. Despite the "enamine" character of VI, the aldehyde group in position 2 is still capable of entering the reaction typical of this moiety. For example, reactions with primary amines lead to the formation of Schiff bases and the interactions with compounds possessing an active methylene group yield 2-vinylindole derivatives. Reaction of VI with malononitrile formed the 2-dicyanovinyl-3-arylaminoindoles VII, which are used to synthesize new indoles and condensed indole derivatives.

Heating compound VII for a short time in acetone in the presence of potassium carbonate leads predominantly to the hydration of vinyl fragment with the formation of initial aldehyde XI. The δ-carboline cyclization dominates when XII is heated in a DMF - MeOH (1 : 1) mixture up to the boiling temperature, and VIII is obtained at a 73% yield. The δ-carboline structure of VIII was confirmed by ^1H NMR spectroscopic data (Ryabova et al., Pharm. Chem. J. 30: 579-584, 1996). The ^1H NMR spectrum of VIII in DMSO-d6 contains the following signals (δ, ppm): 6.17 (bs, 2H), 5.91 (d, I H, H-C^9), 6.74 (t, 1H, H-C^8), 7.23 (t, 1H, H-C^7) and 7.42 (q, 1H, H-C^6).[2)] 7.88 and 8.55 (A_2B_2 system, 4H, $C_6H_4NO_2$), 8.25 (s 1H, H-C^4). A characteristic feature of the latter spectrum is a considerable upfield shift of the H-C^9 proton signal (5.91 ppm) as compared to the signals of other protons of the benzene ring (6.74 - 7.42

ppm) and the analogous proton signals in the spectra of pyrrolo[1,2-a]indole (7.27-7.94ppm) and 3-arylamino-2-formylindole X (6.95-7.59 ppm). Apparently, this shift of the H-C^9 signal toward higher field strengths can be only due to the effect of anisotropic circular currents of the 4-nitrophenyl substituent in position 1, displaced out of the plane of the molecule as a result of steric constraints (the Dreiding molecular models). Thus, the experimental data confirmed the δ-carboline structure of VIII.

Alkylation of 3-aminoindole, initial aldehyde VI, 2-vinyl derivative VII, and 1,2-dihydro-δ-carboline VIII was used to develop a general method for making N-alkyl derivatives. This provided a common approach to obtaining compounds substituted at the exocyclic amino group and the nitrogen atom of the indole cycle. According to the mass-spectrometric data, methylation of VII by methyl iodide in acetone in the presence of potassium carbonate leads to the formation of a mixture of mono- and dimethyl derivatives, 2-formylindole XI, and δ-carboline X. Using column chromatography methods, aldehyde XI was isolated as was a bis-dimethyl derivative from this mixture. A side product in this reaction was 3-(4-nitrophenylamino)indole-2-carboxylic acid.

On heating in the presence of an aqueous alkali with dimethyl sulfate in acetone, compound XIII is methylated at the endo- and exocyclic nitrogen atoms (probably, via the stage of formation of the corresponding anion) yielding δ-carboline X from the reaction mixture (Scheme 3).


NO2
NH
CN
N
H
XIII
NO2
N—CH3
CN
N
CH3
XIV
NO2
CH3
N
N⊕
CH3
CN
N
CH3
NO2
N
N
CN
N
CH3
⊕
⊖CH2-COCH3
O2N
CH3
N
N
CH3
CN
N
CH3
O
CH3
X

Scheme 3

The ^{1}H NMR spectrum of X (Table 2) contains signals from two methyl groups: δ = 3.18 ppm (s. 3H, 2-NMe) and 3.81 ppm (s, 3H. 5-NMe). On saturation of the low-field N-methyl group signal, the intensity of the doublet at δ = 7.45 ppm increases by 8%, and that of the singlet at δ = 8.50 increases by 14%. In contrast, saturation of the signal of the other methyl group leads to no increase in the intensity of signals from aromatic protons. At the same time, saturation of the low-field part (δ =- 7.70 ppm) of the A_2B_2 system of signals from protons of the 4-nitrophenyl fragment increases by 4% the intensity of a doublet (δ = 5.82 ppm) belonging to the proton at C^9. The above NOE estimates unambiguously confirm the proposed structure of compound X, in which the methyl group at N^5 approaches the positions of H-C^4 and H-C^6, while the proton at C^9 is close to protons of the 4-nitrophenyl substituent in position 1. The comparatively small increase in intensity of the doublet due to C_9 protons (δ = 5.82 ppm), observed on saturation of the signal from *ortho* protons of the nitrophenyl fragment, is probably explained by increasing distance to this proton system as a result of displacement of the N^1-aryl substituent out of the molecular plane. This also leads to the upfield shift of the signal from H-C^9.

A different reaction of VIII with methyl iodide is observed in the presence of potassium carbonate, whereby the final result is determined by the methylation medium. For example, prolonged heating of the components in acetone leads to trimethylation of the initial carboline, accompanied by attachment of the acetonyl anion in position 4. As a result, tricyclic XI was obtained (Scheme 3), in which the indole cycle is linked to the 1,4-dihydropyridine ring having a new functional substituent in position 4.

The dimethyl derivative X is apparently an intermediate involved in the formation of the acetonitrile derivative XI. This is confirmed by the fact that methylation of X under the conditions indicated above leads to a 65% yield of XI. A similar pattern is observed if cyclohexanone or methylethylketone are used as solvents instead of acetone: in this case the process leads to 1-(4-nitrophenyl)2-dimethylamino-3-cyano-4-(2-oxocyclohexyl) and (3-oxo-2-butyl)-5-methyl-1,4-dihydro-δ-carbolines, respectively.

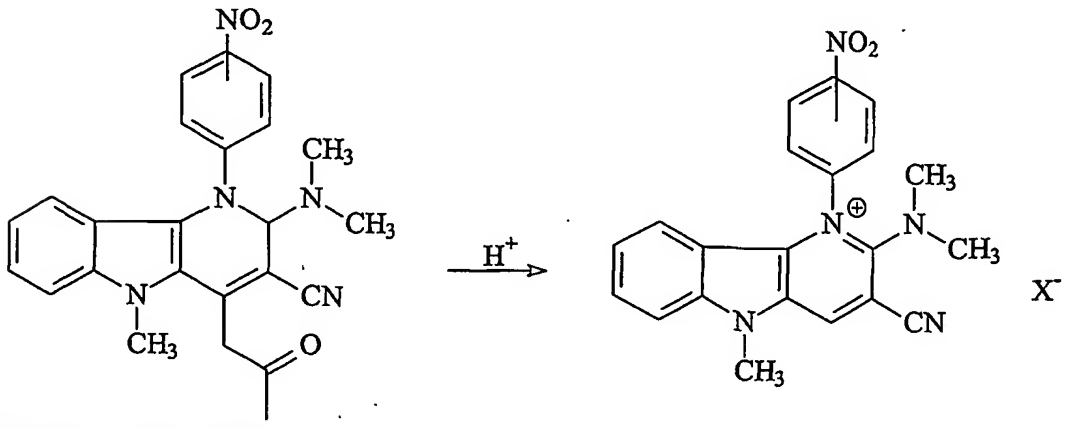
This initial stage may involve exhaustive methylation with the formation of a cation, in which the positive charge is delocalized between a dimethylamino group and position 4 of the molecule. It is this position to which the anion of a ketone (present in the reaction mass) is attached in the following stage with the formation of 1,4-dihydro-δ-carbolines.

The proposed structure of synthesized δ-carbolines was confirmed by spectroscopic data, primarily by the results of NMR measurements. For example, and with reference to compound X, the IR spectrum of this compound, measured as a Nujol mull, showed the absorption bands at 1720 cm^{-1} (nonconjugated ketone) and 2190 cm^{-1} (CN group); mass spectrum *(m/z):* 429 [M^+]; 372 [M^+-CH_2COCH_3]; ^1H NMR spectrum in DMSO-d_6 (δ, ppm): 3.75 (s, 3H, NMe), 2.90 (bs, 6H, NMe), 2.10 (s, 3H, $CH_2CO\underline{CH_3}$), 2.69 (AB-system, 2H, J_{hem} 17 Hz, J^1_{vic} 9 Hz, J^2_{vic} 5 Hz $\underline{CH_2}COCH_3$), 4.31 (q, 1H, H-C^4), 7.89 (A_2B_2-system, 4H, $C_6H_4NO_2$), 7.08 - 7.53 (4H, aromatic protons).

The IR spectra of synthesized compounds were measured on a Perkin-Elmer Model 457 spectrophotometer using samples prepared as Nujol mulls. The mass spectra were obtained on a Varian MAT-112 mass spectrometer with direct introduction of samples into the ion source operated at an ionizing electron energy of 70 eV. The NMR spectra were recorded on a Varian XL-200 instrument (USA) using TMS as the internal standard. The course of reactions was monitored and the samples were identified by thin-layer chromatography on Silufol UV-254 plates eluted in the chloroform methanol system (10:1). The data of elemental analyses coincided with the results of analytical calculations.

III. Conversion of BAIPs, such as Compound 2, into Active Drug

Compound 2 has been found to be a prodrug for forming other BAIPs. For example, exposure of Compound 2 to acidic conditions converts prodrug 2 to active drug 4.



Compound 2 (prodrug) **Compound 4 (active drug)**

Under the same conditions, Compound 2 also may be converted into the dimer compound 6, which is an active drug. Optimal conversion of prodrug to active drug was found to involve treatment of Compound 2 with concentrated HCl (11.6 N) overnight.

Examples 1-3 below provide additional information concerning methods for making the compounds described herein.

IV. Biological Activity of BAIPs

Compound 2 exerts broad anti-retroviral activity and has low cellular toxicity. Compound 2 initially was found active against HIV-1_{RF} in a standard screening cytoprotection assay (EC_{50}=0.1 μM and a CC_{50}>200 μM) that requires multiple rounds of viral infection. Range of action studies showed that Compound 2 also inhibited a panel of retroviruses, including laboratory and clinical isolates of HIV-1, HIV-1 isolates housing mutations that confer resistance to nucleoside and NNRTIs, monotropic and lymphotropic HIV-1 strains, as well as HIV-2 and SIV (Table 1).

Table 1 - Antiviral Properties of Compound 2

Virus	Cell	EC_{50}	CC_{50}	TI
HIV-1 RF	CEM-SS	0.1	>200	>2000
		0.078	>200	>2570
HIV-1 IIIB	CEM-SS	0.824	>200	>242
		0.836	126	151
HIV-1 OC/100	CEM-SS	4.68	116	24.9
		1.19	113	94.5
HIV-1 HEPT/236	CEM-SS	0.97	133	137
HIV-1 CALO-R	CEM-SS	1.10	122	110
		1.14	176	153
HIV-1 ddI-R	CEM-SS	0.62	163	263
HIV-1 DPS-R	CEM-SS	0.5	123	247
HIV-1 4X AZT	CEM-SS	1.3	110	84.8
HIV-1 A-17	CEM-SS	2.98	92.1	30.8
		3.48	88.1	25.3
HIV-1 6R/AZT	CEM-SS	16.6	130	7.8
		12.0	109	9.1
HIV-1 6S/AZT	CEM-SS	1.41	125	
		0.5	68.7	136
HIV-1 N119	CEM-SS	1.01	109	108
		9.73	124	12.8
HIV-2 ROD	CEM-SS	2.64	162	61.1
		4.79	>200	>41.7
		0.37	>200	>539
SIV	CEMx174	5.65	>200	>35.4
		6.5	134	20.6

[1]XTT antiviral assays were performed as described below in Example 4. EC_{50} values indicate the drug concentration that provided 50% cytoprotection. CC_{50} values reflect the drug concentrations that elicit 50% cell death. The XTT cytoprotection studies with HIV-1 were confirmed by measurement of supernatant RT, p24 and infectious virus titers.

Mechanistic studies showed no inhibitory activity of Compound 2 against RT when evaluated *in vitro* with recombinant p66/p51 RT using either the poly(rA) oligo(dT) or poly(rC) oligo(dG) template-primer systems. Likewise, Compound 2 did not affect virus binding or fusion to target cells, the activities of HIV-1 integrase or protease enzymes, or the nucleocapsid protein zinc fingers (Table 2).

Table 2 - Mechanism of Action Studies with Compound 2

Molecular Target[1]	Effect
RT (rAdT and rCdG)	NI[2]
Protease	NI
Integrase	NI
NCp7 Zn fingers	NI
Biological Target	**Effect**
Early Phase HIV-1 Attachment Time Course Assay MAGI Assay	 40% reduction at 100 μM No inhibition of proviral DNA synthesis No reduction in blue cell formation at 200 μM
Late Phase ACH-2 Assay	 1) No reduction of p24 2) 2) Virus protein processing normal (Western blot) 3) Particle morphology normal (EM) 4) Reduction in RT activity in new virions 5) Reduction of infectious title of new virions

[1]Attachment of HIV-1 to CEM-SS cells, binding of gp120 to CD4, and the effects of compounds on HIV-1 RT, PR and NCp7 were quantitated as described below in Examples 4 and 5. [2]NI indicates that no inhibition was observed at the high test concentration (100 μM).

Thus, Compound 2 appeared not to act on any of the classical anti-HIV molecular targets.

The activity of Compound 2 was evaluated using a MAGI, cell-based, early-phase model of infection, described in Example 7. This assay requires virus binding, fusion, reverse transcription, integration of proviral DNA and the expression of Tat protein. Viruses were added to the MAGI cells in the presence or absence of Compound 2, and viral infectivity determined by scoring the number of blue foci. Compound 2 demonstrated no apparent inhibitory action. Since the agent had no effect on these early-phase events, the data suggested it acted during the late phase of infection, after the HIV provirus integrates into the host cell genome.

Compound 2 was evaluated in a late-phase model of HIV-1 replication. This model uses ACH2 cells, which carry a latent HIV-1 infection. In this model, the ACH2 cells are treated with TNF-α, which stimulates HIV-1 replication and virion production. Compound 2 had no effect on viral p24 antigen levels in the ACH2 cell culture supernatant, suggesting that

virions were produced normally (FIG. 1). However, Compound 2 decreased virion-associated RT and viral infectivity levels in the culture supernatants in a concentration-dependent manner (FIG. 1). These observations were confirmed with latently infected U1 cells, chronically infected H9 cells, and other clones of latently infected ACH-2 cells under TNF-α induced or uninduced conditions (data not shown).

With reference to FIG. 1, ACH2 cells were stimulated with recombinant TNF-α in the absence or presence of various concentrations of Compound 2. Cell-free supernatants were collected and evaluated as described in Examples 4-6. Virus-associated p24 antigen (◇) was quantitated by antigen capture assay, RT activity (□) was assessed by a homopolymeric(rA) template-primer system assay, and infectious units (△) were quantitated by titration of the cell-free supernatant on MAGI cells wherein each blue cell represented an infectious unit. Each point represents the mean of triplicate cells from a representative experiment. Cell viability was unaffected at the relatively high test concentration of 200 μM, as assessed by XTT assay.

The MAGI and ACH2 data, taken together, show that Compound 2 acts during the late phase of infection, after the provirus has integrated into the host cell genome. In the ACH2 assay, a drug which acted intracellularly to inhibit HIV replication would reduce the amount of HIV released into the cellular supernatant. HIV virions were apparently being produced in an essentially normal manner, since Compound 2 treatment did not reduce the amount of viral p24 antigen present in the culture supernatant. When the HIV virions were released from the cell into the culture media, they exhibited significant abnormalities. Compound 2-treated cells showed reduced virion-associated RT activity and viral infectivity levels, and the degree to which the activity was reduced was directly related to the concentration of Compound 2.

To further investigate the observed abnormalities, the HIV-1 virions released from Compound 2-treated cells were compared to control in Western blot and protein analysis and electron microscopy. TNF-α stimulated ACH2 cells were treated with either Compound 2 or control solution, and cell-free supernatants were centrifuged to pellet the virus particles. Samples were subjected to Western blot analysis with AIDS patient serum or with polyclonal antiserum to HIV-1 RT protein as shown by FIG. 2. The positions of gp120, $Pr55^{gag}$ precursor polypeptide, p24 capsid (CA) protein, p17 matrix (MA) protein, integrase (IN), the p66 subunit of HIV-1 RT and p51 subunit of HIV-1 RT are indicated in FIG. 2. This analysis revealed a normal complement of fully mature (processed) HIV-1 proteins, including both subunits of the RT protein, in both control and Compound-2-treated supernatant. Electron micrographs of virus

particles were obtained to assess morphological changes in virus particles treated with compounds of the present invention. Electron microscopy revealed no morphologic differences between virions obtained from control and Compound 2-treated cells. Thus, although virions released from Compound 2-treated cells had lower RT activity and were less infectious than virions released from control-treated cells, there were no abnormalities in virion morphology or protein composition that explained the difference.

A. Compound 2 is a Prodrug

The actual mechanism of action of Compound 2 became apparent partially from studies in which virion-associated RT levels were measured following centrifugation of virus particles in the virus-rich ACH-2 culture media. With reference to FIG. 3, RT activity (◦) and infectious units (□) were quantified in the cell-free supernatant from TNF-α stimulated ACH2 cells in the presence of Compound 2. Activity levels decreased as the concentration of Compound 2 increased. A separate set of samples was centrifuged and the fluid phase removed prior to quantifying RT levels (○) and infectious units (❑) of the virus pellet. Removing the culture fluid from the centrifuged virus particles allowed recovery of RT activities and virus infectivity at levels equivalent to those found in virions from untreated ACH-2 cultures (FIG. 3). This indicated that Compound 2 was a prodrug that had been converted into an active and reversible RT inhibitor during the 72-hour culture period. This was confirmed by a study in which the RT activity in a lysate of normal HIV-1 virions was inhibited by addition of virus-depleted culture supernatant from drug-treated ACH-2 cells. In contrast, addition of drug-free culture media or fresh drug to the normal virions did not inhibit their RT activity.

B. Purifying Active Forms of Compound 2

Studies were designed to determine the conditions for converting prodrug 2 into an active form. Efficient conversion of prodrug 2 into active forms was achieved by exposing Compound 2 to acidic, generally aqueous, conditions. Optimal conversion was achieved by exposing Compound 2 to concentrated HCl (11.6N) overnight at ambient temperature.

To establish the chemical nature of the conversion to active form, the acidified preparation of Compound 2 was resolved by high-pressure liquid chromatography (HPLC) and analyzed by mass spectrometry and NMR spectrometry. HPLC resolved two major fractions (Fractions 5 and 11), which were analyzed further. Fraction 5 was a pure compound and

represented the major component of the acid-treated material (about 90%). Fraction 11 also was a pure compound, but represented only a minor component (about 1% of the treated material). Mass spectrometry and NMR spectrometry showed that Fraction 5 was monomeric Compound 4, benzoylmethylindolepyridinium (BMIP). The compound in Fraction 11 was Compound 6 (a dimeric form of BMIP Compound 4).

C. Biological Data for Compounds 4 and 6

BAIP Compound 4 inhibited HIV-1 virion-associated RT with an IC_{50}=9.7 μM (rAdT template-primer system). The dimer, Compound 6, was approximately 50-fold more effective (IC_{50}=0.21 μM) than Compound 4. The XTT cytoprotection assay of Compound 4 showed that its anti-HIV-1 activity (EC_{50}=0.28 μM) was equivalent to the prodrug, while being less toxic (CC_{50}>316 μM). Compound 6 exhibited a similar activity against HIV-1 replication (EC_{50}=0.49 μM), but was more cytotoxic (CC_{50} about 20 μM). Thus, although the dimer, Compound 6, was a more potent RT inhibitor, the disparities in cytotoxic properties focused mechanistic efforts on BMIP Compound 4.

1. Antiviral and mechanistic studies of BMIP, Compound 4

Compound 4 was evaluated for its antiviral activity against various strains of HIV-1, as well as HIV-2, and SIV. HIV-1 strains tested included representatives of Clades A-F, and strains having mutations in RT that confer resistance to known ddN-, ANP-, and NNRTI-class RT inhibitors. As summarized in Table 3, Compound 4 demonstrated antiviral activity against all of these retroviruses.

Table 3 - Range of Antiretroviral Action of Compound 4 (BMIP)

A.			
HIV-1 Isolate	**COMPOUND 4 (μM)**	**Fold resistance**	**AZT (μM)**
IIIB	0.82		0.007
OC/L100I4.73	5.7	0.004	
T/C/K101E	1.34	1.6	0.020
129/C/K103N	0.87	1.1	0.03
Thia/V108I	0.97	1.2	0.02
Calo/T139I	0.88	S	0.02
DPS/Y181C	1.72	2.1	0.003
3TC/M184I	2.36	2.9	0.002
Cost/Y188H	1.20	1.5	0.009
HEPT	1.59	1.9	0.180
B.			
CLADE with PBMC	**COMPOUND 4 (μM)**		
A	0.46		0.008
B	0.077		0.003
C	0.094		0.001
D	0.93		0.002
E	0.12		0.001
F	0.56		0.003
C.			
Virus Range	**COMPOUND 4 (μM)**		**ddC (μM)**
HIV-1IIIB/CEM-SS	0.12		0.04
HIV-1SK1/CEM-SS	0.07		0.03
HIV-1RF/CEM-SS	0.07		0.02
HIV-2ROD/CEM-SS	0.95		0.05
SIV/174Xcem	0.48		0.04

Table 3 Legend

[1]Anti-HIV studies utilizing lymphocyte-derived cell lines were performed using the XTT cytopathicity assay, while antiviral assays utilizing PBMC cultures were performed by measurement of cell-free p24 levels. The XTT cytoprotection studies with HIV-1 were confirmed by measurement of supernatant RT, p24 and infectious virus titers. [2]AZT^R, Pyr^R and Nev^R indicate strains of HIV-1 that are resistant to AZT, pyridinone or nevirapine, respectively.

A number of studies were performed in cell culture to confirm that the antiviral activity of Compound 4 was attributable to its anti-RT activity. Compound 4 had no effect on the viral integrase enzyme, its protease, nucleocapsid zinc fingers (NCp7) or fusion to target cells.

Time course assays also were performed in which BAIP compounds were added to MAGI cell cultures at different times following the addition of viruses to cell cultures (time-of-addition study), or the drug was removed from cultures at different times after the addition of virus (wash-out study). MAGI cells were exposed to HIV-1_{IIIB} in the absence or presence of antiviral agents for two hours, after which the unbound virus was removed by washing of cultures, and cultures were then replenished with either drug-free or drug-containing medium.

Under the time-of-addition conditions, FIG. 5, the BAIPs were added to cultures at the indicated time points, while under the time of removal experiments, the BAIP compounds were removed from cultures and the cultures were washed and replenished with fresh drug-free medium. After 48 hours post-exposure to virus the cells were fixed and stained, and the number of blue cells quantitated with each blue cell representing an infectious unit. The number of blue cells in the drug-free control was set as a value of 100% and the number of blue cells from cultures treated with nevirapine (3.16 μM), dextran sulfate (10 μg/ml) or BAIP compounds (e.g., Compound 4, 10 μM) are expressed as a percentage of the drug-free control value.

As shown in FIG. 5, dextran sulfate, a known inhibitor of virus binding to cells, no longer inhibited if its addition was delayed two hours or more post-infection. In the washout study, the inhibitory effect of dextran sulfate was maintained even if it was washed away during the very early phases of infection. In contrast, the NNRTI nevirapine maintained significant antiviral activity (FIG. 5) even if its addition was delayed for eight hours after infection. In the wash out experiments, nevirapine lost activity only if it was removed during the first four hours post-infection (during the reverse transcription phase). This indicates that nevirapine is a reversible RT inhibitor. The behavior of the BAIPs in these time-of-addition and washout assays resembled that of nevirapine.

Nascent proviral DNA synthesis was examined during the time-of-addition study (data not shown). DNA was purified 4 hours post infection of MAGI cells with HIV-1 in the presence or absence of BMIP and the presence of proviral DNA was evaluated by PCR amplification. Proviral DNA was readily detectable in the absence of inhibitors, while its synthesis was not detected in cultures treated with either nevaripine or BMIP. Thus, BMIP exhibited the biological characteristics similar to an NNRTI, with respect to HIV-1 RT.

2. Characterization of the Effect of BAIP Compound 4 on RT

Compound 4 was distinct from all other NNRTIs because it not only inhibited HIV-1, but also HIV-2 and SIV replication in cell cultures. Compound 4 also inhibited virion-associated RT from HIV-1, HIV-2 or SIV, while nevaripine only inhibited HIV-1 RT (Table 4).

Table 4

Action of Agents Against Polymerase Activities of Reverse Transcriptase Enzymes

I. BMIP with Different Templates and Recombinant HIV-1 RT

	Homopoly RNA		Homopoly DNA		
	rAdT	RCdG	dAdT	dCdG	LTR RNA
BAIP	15.0	NI	56.0	NI	22.34
Nevirapine	1.4	0.11	>10	0.44	0.074

II. BMIP and Nevirapine with Virion-Associated RT (RNA vs. DNA template)

	RNA				DNA			
	BAIP		Nevirapine		BAIP		Nevirapine	
Virus	rAdt	rCdG	rAdt	RCdG	dAdt	dCdG	dAdT	dCdG
HIV-1	9.68	NI	63.5	10.0	21.8	NI	5.44	45.28
HIV-2	51.14	NI	NI	NI	100	NI	NI	NI
SIV	53.2	NI	NI	NI	100	NI	NI	NI

III. BMIP and Nevirapine with Virion-Associated RT (HIV-1 LTR RNA template)

Virus	BAIP	Nevirapine
HIV-1	70.3	18.1
HIV-2	53.2	NI
SIV	76.2	NI

These findings prompted investigations concerning the effect of Compound 4 on the various functions of RT. Compound 4 was examined to determine its ability to inhibit HIV-1 virion-associated RT using a template other than the homopolymeric(rA) (which was used to examine virion-associated RT activity). Compound 4 inhibited both RNA- and DNA-dependent DNA polymerase activities of HIV-1 RT (Table 4). When heteropolymeric HIV-1 LTR RNA was used as the template, Compound 4 also inhibited HIV-1 RT polymerase activity (Table 4). Compound 4 did not inhibit RT polymerase activity if the template used was heteropolymeric(C), no matter if it was RNA or DNA. Finally, Compound 4 did not inhibit HIV-1 RNaseH activity of recombinant HIV-1 RT (data not shown).

V. Molecular Modeling of BAIP and its Dimeric Form with the HIV-1 Reverse Transcriptase Enzyme

Computational analysis of candidate binding sites on the RT protein identified the location of the most probable binding region for the monomeric and dimeric forms of BAIP to be located in the finger subdomain of the p66 monomer of HIV-1 RT. The location of this region is in a pocket clearly formed under the "knuckle" of the most extended finger (residue number 60-75) in this subdomain. The conformation of the test ligand, which was found to computationally bind HIV-1 RT with the greatest binding strength, was positioned at the base of this finger. In this docked position, strong hydrophobic and hydrophilic contacts were made with HIV-1 RT that involved residues K65, R72, K73, L74, V75 and F116. Examination of the crystallographic thermal factors associated with these regions shows that the positions of these side chains are well determined. Superposition of four different crystallographic coordinate sets of HIV-1 RT shows that this binding "pocket" is highly preserved. The location of this binding site involved interactions with residues that do not appear on the list of resistant mutants that arise following therapy with any reported nucleoside and non-nucleoside RT inhibitors. These data indicate that the disclosed BAIP compounds bind to a previously unidentified pocket on the HIV-1 RT enzyme that appears to be shared among many retroviruses. Thus, the BAIPs represent an entirely new class of RT inhibitors.

VI. Summary

The data presented herein show that the BAIPs are a novel class of RT inhibitors with truly broad-spectrum activity against retroviral RT enzymes and against infection by a broad

range of retroviruses, including HIV-1, HIV-2 and SIV. BAIPs demonstrated antiviral activity against laboratory isolates of HIV-1 and a panel of clade-representative clinical isolates in PBMC cultures at submicromolar levels. More impressive though was the ability of the BAIPs to inhibit the replication of a panel of HIV-1 variants carrying mutations in RT that confer resistance to AZT and various NNRTIs, such as oxithiin carboxanilide (L-100→I), thaizolobenzimidazole (V-108→I), calanolode (T-139→I), diphenylsulfone (Y-181→I), 3TC (M-184→I) and others. The ability of the BAIPs to inhibit the enzymatic RT activities and replication of this wide array of retroviruses distinguished it from classical NNRTI-type molecules that are HIV-1 specific and can be typically rendered ineffective by one or more single mutations in the HIV-1 RT enzyme. Thus, the BAIPs truly represent the first reported example of a broadly antiretroviral NNRTI (BANNRTI).

The BAIP compounds have been found to inhibit not only all strains of HIV-1 tested, but also the replication of HIV-2 and SIV. This property sets the BAIPs apart from other NNRTI-type agents. The BAIPs may be used for therapy to individuals already carrying HIV-1 variants that are resistant to AZT or classical NNRTI molecules.

Classical NNRTIs bind noncovalently to the non-substrate binding site of the RT enzyme, and mutations in this region of the enzyme result in loss of sensitivity to the agents. Likewise, nucleoside analogs interact with RT in the substrate binding pocket, and mutations in this region of the enzyme result in resistance to the respective nucleoside analogs. Because BAIPs exert such distinct antiviral properties from the classical NNRTIs and have such a different structure from nucleoside analogs, BAIPs likely interact with RT in a different manner than classical NNRTIs. A series of computational studies were performed that predict the most likely binding site for BAIPs. Such studies suggested that BAIPs (both monomeric and dimeric forms) bound tightly in a previously unidentified pocket near the Asp triad in the active site of the RT enzyme. Together, these studies set the BAIP molecules apart as a new class of RT inhibitors.

VII. Pharmaceutical Compositions Comprising BAIPs

The vehicle in which the drug is delivered can include pharmaceutically acceptable compositions of the drugs, using methods known to those of ordinary skill in the art. Any of the common carriers, such as sterile saline or glucose solution, can be used with the compounds provided by the invention. Routes of administration include, but are not limited to, oral and

parenteral routes, such as intravenous (iv, a currently preferred method), intraperitoneal (ip), rectal, topical, ophthalmic, nasal, transdermal, and combinations thereof.

The drugs may be administered intravenously in any conventional medium for intravenous injection, such as an aqueous saline medium, or in blood plasma medium. The medium also may contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, lipid carriers such as cyclodextrins, proteins such as serum albumin, hydrophilic agents such as methyl cellulose, detergents, buffers, preservatives and the like. A more complete explanation of parenteral pharmaceutical carriers can be found in *Remington: The Science and Practice of Pharmacy* (19th Edition, 1995) in chapter 95. The compositions are preferably in the form of a unit dose in solid, semi-solid and liquid dosage forms such as tablets, pills, powders, liquid solutions or suspensions.

VIII. Administering BAIPs

The present invention provides a treatment for HIV and SIV disease, perhaps by RT inhibition, and associated diseases, in a subject such as a mammal, for example a monkey or human. The method includes administering a compound, or compounds, of the present invention, or a combination of the compound and one or more other pharmaceutical agents. The compound, or compounds, can be administered to the subject in a pharmaceutically compatible carrier. The compound, or compounds, are administered in amounts effective to inhibit the development or progression of HIV and SIV disease. Although the treatment can be used prophylactically in any patient at significant risk for such diseases, subjects can also be selected using more specific criteria, such as a definitive diagnosis of the condition.

The compounds of the present invention are ideally administered as soon as possible after potential or actual exposure to HIV infection. For example, once HIV infection has been confirmed by laboratory tests, a therapeutically effective amount of the drug is administered. The dose can be given by frequent bolus administration.

Therapeutically effective doses of the compounds of the present invention can be determined by one of skill in the art. For example, effective doses can be such as to achieve tissue concentrations that are at least as high as the EC_{50}. The low cytotoxicity of the BAIP makes it possible to administer high doses, for example 100 mg/kg, although doses of 10 mg/kg, 20 mg/kg, 30 mg/kg or more are contemplated. Thus, the dosage range likely is from about 0.1

to about 200 mg/kg body weight orally in single or divided doses, more likely from about 1.0 to 100 mg/kg body weight orally in single or divided doses. For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 1.0 to about 1000 mg of the active ingredient. Symptomatic adjustment of the dosage to the subject being treated can be achieved by suing tablets of varying amounts of compound, such as 1, 5, 10, 15, 20, 25, 50, 100, 200, 400, 500, 600, and 1000 mgs of the active ingredient.

The specific dose level and frequency of dosage for any particular subject may be varied and will depend upon a variety of factors as will be known to a person of ordinary skill in the art. These include the activity of the specific compound, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, and severity of the condition of the host undergoing therapy.

The pharmaceutical compositions can be used in the treatment of a variety of retroviral diseases caused by infection with retroviruses that require reverse transcriptase activity for infection and viral replication. Examples of such diseases include HIV-1, HIV-2, and the simian immunodeficiency virus (SIV).

The present invention also includes combinations of a BAIP compound, or BAIP compounds, of the present invention with one or more agents useful in the treatment of HIV disease. For example, the compounds of this invention may be administered, whether before or after exposure to the virus, in combination with effective doses of other antivirals, immunomodulators, anti-infectives, or vaccines. The term "administration" refers to both concurrent and sequential administration of the active agents.

Examples of antivirals that can be used in combination with the BAIP RT inhibitors of the invention are: AL-721 (from Ethigen of Los Angeles, CA), recombinant human interferon beta (from Triton Biosciences of Alameda, CA), Acemannan (from Carrington Labs of Irving, TX), ganciclovir (from Syntex of Palo Alto, CA), didehydrodeoxythymidine or d4T (from Bristol-Myers-Squibb), EL10 (from Elan Corp. of Gainesville, GA), dideoxycytidine or ddC (from Hoffman-LaRoche), Novapren (from Novaferon labs, Inc. of Akron, OH), zidovudine or AZT (from Burroughs Wellcome), didanosine, lamiduvine, delavirdine, nevirapine, ribavirin (from Viratek of Costa Mesa, CA), alpha interferon and acyclovir (from Burroughs Wellcome), indinavir (from Merck & Co.), 3TC (from Glaxo Wellcome), Ritonavir (from Abbott), Saquinavir (from Hoffmann-LaRoche), nelfinavir, and others.

Examples of immunomodulators that can be used in combination with the BAIPs of the invention are AS-101 (Wyeth-Ayerst Labs.), bropirimine (Upjohn), gamma interferon (Genentech), GM-CSF (Genetics Institute), IL-2 (Cetus or Hoffman-LaRoche), human immune globulin (Cutter Biological), IMREG (from Imreg of New Orleans, La.), SK&F106528, and TNF (Genentech).

Examples of some anti-infectives with which the BAIPs can be used include clindamycin with primaquine (from Upjohn, for the treatment of pneumocystis pneumonia), fluconazlone (from Pfizer for the treatment of cryptococcal meningitis or candidiasis), nystatin, pentamidine, trimethaprim-sulfamethoxazole, and many others.

The combination therapies are not limited to the lists provided, but include any composition for the treatment of HIV disease and related retroviral diseases (including treatment of AIDS).

IX. EXAMPLES

The following examples are provided to exemplify certain particular features of working embodiments of the present invention. The scope of the present invention should not be limited to those features exemplified.

Example 1

This example describes methods for making Compound 2 and related compounds.

2-Cyano-3-[3-(4-nitrophenylamino)-2-indolyl]acrylic acid nitrile (VII, Scheme 1).

Method 1. A mixture of 3.65 g (13 mmole) of compound VI, 1.6 g (24 mmole) malononitrile, 0.25 ml (2 mmole) triethylamine, and 73 ml of 2-propanol was stirred for 5 h at 20°C and allowed to stand at this temperature for 16 h. The precipitate was separated by filtration and washed with 2-propanol to obtain 3.3 g of VII.

Method 2. A mixture of 3 g (11 mmole) of Compound VI, 1.5 g (22 mmole) malononitrile, and 60 ml of 2-propanol was refluxed for 4 h and allowed to stand for 16 h at 20°C. Then the reaction mixture is treated as in method 1 to obtain 2.7 g of VII.

Method 3. A suspension of 0.3 g (1 mmole) of N-acetylated derivative of VI, 0.1 g (1.5 mmole) malononitrile, and 0.13 g (1.5 mmole) fused sodium acetate in 5 ml of acetic acid was stirred for 0.5 h at 20°C, followed by 3 h at 80°C. Then 0.1 g of malononitrile was added and the mixture was stirred for another 5 h at 20°C. Then the mixture was cooled, and the

precipitate was separated by filtering and washed with AcOH, water, and MeOH to obtain 0.05 g of VII.

1-(4-Nitrophenyl)-2-imino-3-cyano-1,2-dihydro-5H-pyrido[3,2-b]-indole (XIII, Scheme 3).

Method 1. A mixture of 3.3 g (10 mmole) of nitrile VII (Scheme 1), 15 ml MeOH, and 15 ml DMF was heated to boiling. As a result, VII dissolved and a new precipitate appeared. This suspension was boiled for 5 min and cooled. The precipitate was separated by filtering and washed with MeOH to obtain 2.4 g of XIII (Scheme 3). ^{13}C NMR spectrum in DMSO-d_6 (δ, ppm): 154.9 (C^2), 99.8 (C^3), 133.9 (C^4), II 9.8 (C^{4a}), 114.5 (C^{9b}). 139.9 (C^{5a}), 128.8 (C^{9b}), 113.1, 119.9. 126.2, 127.1 (C^6-C^9), 119.9, 131.1 ($C^{2'}$, $C^{3'}$, $C^{5'}$, $C^{6'}$,). 148.1, 144.1 ($C^{1'}$ $C^{4'}$), 117.7 (CN).

Method 2. A mixture of 0.33 g (1 mmole) of nitrile VII and 0.4 g (3 mmole) of calcined potassium carbonate in 10 ml of acetone was boiled for 15 min. The precipitate was separated by filtering and washed with water to obtain 0.05 g of XIII. The acetone mother liquor was evaporated, and the residue triturated with diethyl ether to obtain 0.17 g (61%) of VI (Scheme 1).

1-Amino-4-oxo-2-cyano-4H-pyrrolo[1,2-a]indole. A mixture of 0.25 g (1 mmole) of 2-cyano-3-(3-acetoxy-2-indolyl) acrylic acid, 10ml MeOH, and 0.15ml (1.5 mmole) triethylamine was refluxed for 5 h and cooled. The precipitate was separated by filtering and washed with MeOH to obtain 0.1 g of 1-Amino-4-oxo-2-cyano-4H-pyrrolo[1,2-a]indole.

1-Methyl-2-formyl-3-[N-methyl-N-(4-nitrophenyl)amino]-indole.

Method 1: To a mixture of 0.33 g (1 mmole) of nitrile VII and 0.4 g (3 mmole) of calcined potassium carbonate in 10 ml of acetone was added 2 ml methyl iodide (MeI) and the mixture was boiled for 20 h, with 2 ml MeI added each 5 h. Then the mixture was cooled and the remaining potash separated by filtering. The filtrate was evaporated, and the residue dissolved in 20 ml of boiling 2-propanol. The solution was filtered and evaporated, and the residue chromatographed on a silica gel column with chloroform. Sequential 50-70 ml fractions were collected and analyzed by TLC. The fractions containing individual products (1 and 5-8) were evaporated. Fraction 1 yielded 1-Methyl-2-formyl-3-[N-methyl-N-(4-nitrophenyl)amino]-indole (m.p., 135°C; M^+, 309), and combined fractions 5-8 yielded VI (m.p., 230°C; decomp.).

Method 2. To a mixture of 0.56 g (2 mmole) of compound VII and 0.83 g (6 mmole) of calcined potassium carbonate in 20 ml of acetone was added 2 ml MeI and the mixture was boiled for 30 h, with 2 ml MeI added each 7 h. Then the remainder of potash was separated by

filtering, the filtrate was evaporated, and the residue chromatographed on a silica gel column with chloroform. Three sequential fractions of 250, 40, and 130 ml were collected, and the first of these used to obtain 0.5 g of 1-methyl-2-formyl-3-[N-methyl-N-(4-nitrophenyl)amino]indole having the melting point IR, and ^{1}H NMR spectra identical with those of the final product of method 1. The third fraction was re-chromatographed on the silica gel column and the same three fractions were collected. The second of these fractions was evaporated, and the residue triturated with diethyl ether on adding 3 drops of MeOH. The precipitate was separated by filtering to obtain 0.02 g of 2-carboxy-3-(4-nitrophenylamino)indole.

Methylation of 3-(4-nitrophenylamino)indole. To a mixture of 1.3 g (5 mmole) of 3-(4-nitrophenylamino)indole, 16 ml DMF, and 2.1 g (15 mmole) of calcined potassium carbonate was added 5 ml MeI and the mixture was stirred at 80°C for 60 h, with 2 ml MeI added each 6 h (to a total of 20 ml). Then the mixture was cooled, the remainder of potash separated by filtering and washed with DMF, and the filtrate was evaporated. The residue was triturated with diethyl ether on adding a minimum amount of MeOH and filtered. The filtrate was evaporated, and the residue chromatographed on a silica get column with chloroform. Five sequential 100 ml fractions were collected, and the third and fifth fractions containing individual products were evaporated. Fraction 1 yielded 0.6 g (42%) of 1-methyl-3-[N-methyl-N-(4-nitrophenyl)amino]indole, and fraction 3 yielded 0.4 g of 3-[N-methyl-N-(4-nitrophenyl)amino]indole.

1-(4-Nitrophenyl)-2-methylimino-3-cyano-5-methyl-1,2-dihydro-5H-pyrido[3,2-b]indole (XIV, Scheme 3). To a solution of 2 g (50 mmole) of NaOH in 2 ml water was added 100 ml acetone and 3.3 g (10 mmole) of XIII, and the mixture was heated to boiling on stirring and boiled for 5 min. To this mixture was added 4 ml (40 mmole) of Me_2SO_4 and the boiling was continued with stirring for 6 h. Another 4 ml of Me_2SO_4 was added and the mixture was boiled for another 6 h. Then the mixture was cooled, the precipitate separated by filtration, washed with acetone, and dissolved in 500 ml of boiling water. The solution was filtered hot, cooled and alkalified with 1N KOH (15 ml). The precipitate was filtered and washed sequentially with water, 2-propanol, and diethyl ether to obtain 2.1 g of XIV.

1-(4-Nitrophenyl)-2-dimethylamino-3-cyano-4-(2-oxo-propyl)-5-methyl-1,4-dihydro-5H-pyrido-[3,2-b]indole (X, Schemes 2 and 3)

X is identical to the Compound 2 prodrug described in this application.

Method 1. To a suspension of 2.15 g (6.5 mmole) of X111 and 3.6 g (26 mmole) of calcined potassium carbonate in 80 ml of acetone was added 2 ml MeI and the mixture was refluxed on stirring for 60 h, with 2 ml MeI added each 7 - 8 h. Then the mixture was cooled and the remaining potash separated by filtering and washed with acetone. The filtrate was evaporated, and the residue triturated with water, filtered, and washed with water and methanol to obtain 2.1 g of a technical-purity product 1-(4-nitrophenyl)2-dimethylamino-3-cyano-4-(oxo-propyl)-5-methyl-1,4-dihydro-5H-pyrido[3,2-b]indole. The product was purified by boiling with 20 ml MeOH, after which the insoluble precipitate was filtered to obtain 1.5 g of compound 1-(4-nitrophenyl)-2-dimethylamino-3-cyano-4-(oxo-propyl)-5-methyl-1,4-dihydro-5H-pyrido[3,2-b]indole.

Method 2. A mixture of 1.07 g (3 mmole) of XIV, 0.83 g (6 mmole) calcined potassium carbonate, 70 ml acetone, and 2 ml MeI was boiled with stirring for 45 h, followed by a procedure similar to that in method 1. This yielded 0.85 g of X, which was identical to the product obtained by method 1.

1-(4-Nitrophenyl)2-dimethylamino-3-cyano-4-(oxo-cyclohexyl)-5-methyl-1,4-dihydro-5H-pyrido[3,2-b]indole. This compound was obtained similarly to X, by treating a mixture containing 0.33 g (1 mmole) of compound XIII, 0.55 g (4 mmole) potassium carbonate, 10 ml cyclohexanone, and 2 ml MeI at 60°C for 40 h. The filtrate was evaporated, the residue dissolved in chloroform, and the solution filtered and evaporated. The new residue was triturated with water, and the precipitate was filtered and washed with water and methanol to obtain 0.37 g of 1-(4-nitrophenyl)2-dimethylamino-3-cyano-4-(2-oxo-cyclohexyl)-5-methyl-1,4-dihydro-5H-pyrido[3,2-b]indole.

1-(4-Nitrophenyl)-2-dimethylamino-3-cyano-4-(2-oxo-2-butyl)-5-methyl-1,4-dihydro-5H-pyrido-[3,2-b]indole. To a suspension of 0.33 g (1 mmole) of XIII and 0.65 g (4.7 mmole) of calcined potassium carbonate in 20 ml of methylethylketone was added 2 ml MeI . The mixture was boiled with stirring for 41 h, with 2 ml MeI added each 6 h. The mixture was cooled and the remaining potash separated by filtering and washed with diethyl ether, water, and methanol. The residue was mixed with chloroform and the solution filtered and evaporated. The residue was triturated with ether, and the precipitate was filtered and washed with ether to obtain 0.1 g of 1-(4-nitrophenyl)-2-dimethylamino-3-cyano-4-(2-oxo-2-butyl)-5-methyl-1,4-dihydro-5H-pyrido-[3,2-b]indole.

Example 2

This example describes a method for making Compound 4. Compound 2 was treated with 6N HCL. Purification of the active fractions was performed by HPLC separation. Fraction 5 of the HPLC effluent was determined by mass spectrometry and NMR analyses to be Compound 4.

Example 3

This example describes a method for making Compound 6. Treatment of Compound 2 with 6N HCL formed new molecular species. Purification of the active fractions was performed by HPLC separation. Fraction 11 of the HPLC effluent was determined by mass spectrometry and NMR analyses to be Compound 6.

Example 4

This example describes virus replication inhibition assays that have been performed. The established human cell lines and laboratory-derived virus isolates (including drug resistant virus isolates) used in these evaluations have previously been described (Weislow et al., 1989; Rice and Bader, 1995). The antiviral activities and toxicity profiles of the compounds were evaluated with CEM-SS cells and HIV-1_{RF} using the XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide) cytoprotection microliter assay which quantifies the ability of a compound to inhibit virus-induced cell killing or to reduce cell viability itself (Weislow et al., 1989; Rice and Bader, 1995). The data are reported as the concentration of drug required to inhibit 50% of virus-induced cell killing (EC_{50}) and the concentration of drug required to reduce cell viability by 50% (CC_{50}). HIV-1 isolates utilized included common laboratory strains (RF, IIIB and MN), as well as a panel of HIV-1 clinical isolates (Rice et al., 1997). The pyridinone-resistant HIV-1_{A17} isolate was obtained from Emilio Emini at Merck Sharpe and Dohme Laboratories. CEM, U1, ACH-2, HeLa-CD4-LTR-β-gal, 174xCEM, and H9/HTLV-IIIB NIH 1983 cell lines were obtained from the AIDS Research and Reference Reagent Program (National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD), as were the HIV-2_{ROD} and the SIV isolates. Phytohemagglutinin-stimulated human peripheral blood lymphocytes and monocyte/macrophages were prepared and utilized in antiviral assays as previously described

(Rice et al., 1996), and levels of virion-associated p24 in cell-free culture supernatants were determined via antigen capture ELISA (Beckman Coulter).

Example 5

This example describes integrase, protease, RT and NC zinc finger assays that have been performed. *In vitro* inhibitory activity against recombinant HIV-1 protease was performed with a reverse-phase high-pressure liquid chromatography assay utilizing the Ala-Ser-Glu-Asn-Tyr-Pro-Ile-Val-Glu-amide substrate (multiple Peptide System, San Diego, CA.) (Rice et al., 1993a). The *in vitro* actions of compounds on 3'-processing and strand transfer activities of recombinant HIV-1 integrase were assayed according to Bushman and Craigie (1991), but with modifications (Turpin et al., 1998). The action of compounds on the RNA-dependent polymerase activity of recombinant HIV-1 p66/p51 RT was determined by measuring incorporation of [^{32}P]TTP or [^{32}P]GTP into the poly rA:oligo dT(rAdT) or poly rC:oligo dG(rCdG) homopolymer template-primer systems, respectively, while the inhibition of drug on the DNA-dependent polymerase activity of purified recombinant HIV-1 RT was determined by measurement of incorporation of [^{32}P]TTP or [^{32}P]GTP into the polydA:oligodT)dAdT) or polydC:oligodG(dCdG) homopolymer template-primer systems, respectively (Pharmacia Biotech, Piscataway, NJ). Reactions were performed in the presence or absence of the drug as described previously (Rice et al., 1997). Reactions were terminated with ice-cold 10% trichloroacetate, filtered through GF/C filter under vacuum, and the filters were then washed with 100% ethanol and [^{32}P] incorporation quantitated by Cerenkov counter. The LTR region of the HIV-1 gemonic RNA was prepared from a pGEM LTR by in vitro transcription with T7 RNA polymerase (Promega, Madison, WI). In pGEM LTR, LTR region from pNL4-3 was inserted into the polyliker of pGEM (Promega) in the orientation that the sense LTR RNA were made when T7 RNA polymerase was used. The rest of steps for the preparation of heteropolymeric primer-template and RT reaction was performed as described (Gu et al., 1993).

Virion-associated RT activity was performed as described previously (REF) in the presence or absence of compound with the homopolymeric template-primer (rAdT, rCdG, dAdT and dCdG) (Pharmacia Biotech, Piscataway, NJ) or heteropolymeric template-primer prepared as described above. HIV-2_{ROD10} and SIV virions were obtained by transfection of proviral DNA into HeLa cells.

Example 6

This example describes RNase H cleavage assays that have been performed. An α-[^{32}P]-uridine-labeled RNA template (81 nucleotides in length) was hybridized to a 20-base DNA oligonucleotide in the presence of 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 2.0 mM dithiothreitol, 100 μg/ml acetylated bovine serum albumin, and 10 mM CHAPS as previously described (Gao et al., 1998). For these reactions, 100 ng of RNA (approximately 50,000 cpm) and 20 ng ofDNA (oligonucleotide 3352, 5'TTCTCGACCCTTCCAGTCCC 3') were utilized. Purified HIV-1 wild type RT (45 ng) was mixed with COMPOUND 4 such that the final concentrations were 0.1, 1.0, 10 or 100 μM, and the reactions were initiated by the addition of 60 mM $MgCl_2$ and the annealed RNA/DNA complex in a final volume of 12 μL. This mixture was incubated at 37°C for 1 minute with Compound 4 or for various times without the compound. Reactions were terminated by the addition of 2X loading buffer, and the products were heat denatured and resolved on a 15% denaturing polyacrylamide-7M Urea gel in TBE buffer at 1600 Volts for approximately 90 minutes. Gels were dried and exposed for autoradiography overnight, and the film was developed with a Kodak RP X-OMAT processor.

Example 7

This example describes MAGI cell assays that have been performed. The MAGI cell indicator line was obtained from the AIDS Research and Reference Program, Division of AIDS, National Institute of Allergy and Infectious Disease. MAGI cells are a HeLa cell line that both expresses high levels of CD4 and contains a single integrated copy of a beta-galactosidase gene under the control of a truncated human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR). These cells maintained in DMEM medium supplemented with 5% fetal bovine serum (FBS), 100U of penicillin G sodium, 0.1 mg of streptomycin sulfate, 0.2 mg G418 sulfate, and 0.1 mg of hygromycin B per ml.

We used MAGI cells and an HIV-1 *env*- and *Tat*-expressing HeLa (HL2/3) cell line to perform a fusion assay. *Tat* activates gene expression from the HIV LTR, and therefore upon fusion of MAGI and HL2/3 cells, *tat* expressed in HL2/3 cells (Ciminale et al., 1990) would activate β-galactosidase expression in MAGI cells. MAGI or HL2/3 cells (2.5×10^5 in 500 μl 5%FBS/DMEM) were preincubated with the tested compound for 1 hour at 37°C, respectively. At the end of preincubation, two cell lines were mixed at 1:1 ratio and were continued incubated for another 16 hours. The cells were then fixed and stained for the expression of β-

galactosidase with indolyl-β-D-galatopyranoside (X-Gal) as described previously (Kimpton and Emerman, 1992). The numbers of blue cells were counted by light microscopy.

MAGI cells were also used to examine the effects of compounds on virus replication, from attachment through early gene expression. In these assays, the LTR-driven β-galactosidase gene in MAGI cells would not be activated until the incoming virus had penetrated the cell, reverse transcribed its RNA genome, generated the double-stranded proviral DNA, integrated the proviral DNA into the host cell genome, and expressed its *tat* gene. The assay was preformed as previously described with modifications (Howard et al., 1998). The virus stock used in the assay was prepared either from TNF-α-induced U1 cells (HIV_{IIIB}) or pNL4-3 - transfected from HeLa cells transfected with the pNL4-3 plasmid containing HIV-1 proviral DNA. Viruses were diluted in 200 µl DMEM medium supplemented with 5% fetal bovine serum (FBS), and were titrated to generate approximately 300 blue cells per well in 24 well plates. Viruses were added to the MAGI cells in the presence or absence of the test compound. After 2 hours incubation at 37°C, the virus was removed, the cells were washed and 1 ml 5%FBS/DMEM medium with or without the test compound was added to the cells. For the time-of-addition assay, the compound was added at time zero when the infection was initiated, or at 2, 4, 8 or 24 hours post initiation of the infection. For the time-of-removal assay, the compound was added to all wells at the beginning of infection and was then removed at 2, 4, 8, 24 or 48 hours thereafter. The cells were washed once with medium after removal of the drug followed by the readdition of 1ml 5%FBS/DMEM fresh medium. Forty-eight hours post initiation of infection, cells were fixed and stained as described above.

To titrate the infectivity of viruses harvested from the drug-treated chronic infected cells, MAGI cells were also used. Either 500 µl total culture media or 200 µl pelleted viruses were added to the 24 well culture plates in the presence 20 µg/ml DEAE-dextran for 3 hours at 37°C prior to the addition of 2 ml of media. The cultures were fixed and stained as described above.

Example 8

This example describes PCR analysis of nascent proviral DNA. MAGI cells were plated at a density of $4x10^5$/well in a 6-well plate. Twenty-four hours later, the cells were infected with HIV_{IIIB} viruses in 500 µl 5%FBS/DMEM in the presence or absence of the compound. HIV_{IIIB} viruses were prepared from TNF-α-induced U1 cells and the amount used

in one infection was titrated as the amount producing 1000 blue colonies. Four hours post-infection, the cells were trypsinized, washed and digested at 55°C for 1 hour with 100 μg/ml protease K in 100 μl buffer containing 0.5% Triton X-100, 100 mM NaCl, 50 mM Tris (pH 7.4), and 1 mM EDTA. To inactivate protease K, the samples were then heated at 100°C for 15 minutes. PCR reactions were performed using M661 and M667 primers (Zack et al., 1990) and 5 μl sample was used in each reaction.

Example 9

This example describes ACH2 latently-infected cell assays that have been performed. ACH2 cells were maintained in RPMI 1640-10% FBS medium. Forty thousand ACH2 cells per milliliter were induced with 5 ng of recombinant tumor necrosis factor alpha (TNF-α) (Sigma Chemical Co., St. Louis, MO) per ml for 24 hours. Twenty-four hours later, an equal volume of medium supplemented with 5 ng of TNF-α per ml and with the appropriate (2x final) concentration of the tested compound was added to cells. Viruses containing cell-free supernatants were collected 48 hours later, and they were subjected directly or after being pelleted through centrifugation for RT assay, p24 assay, and virus titration assay. Viability of the cultures was determined by XTT dye reduction). The RT assay, virus titration assay with MAGI cells, and p24 assay were performed as described above.

Pelleted virus particles were also subjected to Western blot analysis. The virion-associated viral proteins pelleted from 400 μl of cell free supernatant were resolved on 10% SDS-polyacrymide gels, were electroblotted onto polyvinylidene difluoride (PVDF) membranes, and were detected by AIDS patient sera or by rabbit-polyclonal anti-HIV-1 RT antibody (AIDS Research and Reference Program, Division of AIDS, National Institute of Allergy and Infectious Disease). Western blots were developed with standard methodology by chemiluminescence (Dupont-NEN, Wilmington, Del.) with a goat-anti human or goat anti-rabbit horseradish peroxidase-conjugated antibody (Bio-Rad, Hercules, Calif.).

Example 10

This example describes molecular modeling that has been done concerning BAIPs. The following analysis was carried out on the HIV-1 RT coordinates 1RTH (Abola et al., 1987: Bernstein et al., 1977). A two-stage analysis was performed. First, the exterior surface of the HIV-1 RT heterodimer was probed for candidate binding regions. This process consists of

localized sampling of the solvent accessible surface to determine a statistical probability that a candidate ligand may bind at this site. The model used to make the calculation has been parameterized, based on a broad sampling of protein-ligand crystal complexes available in the Brookhaven database of protein structures. (PDB) (Abola et al., 1987; Bernstein et al., 1977). The complete details for identification of putative protein binding sites can be found in Young et al. (Young et al., 1994). Second, the optimal docked position of the test ligand was determined. Families of possible conformations for the test ligand were generated using standard modeling techniques and each was docked to the regions defined in the first step. The docking procedure has been demonstrated to have an accuracy of within 1Årms deviation from the known docked positions (Wallqvist & Covell, 1996). The position of the ligand with the strongest calculated binding strength is reported herein.

Example 11

This example describes the preparation of samples for electron microscopy. Sample preparation for electron microscopy is described previously (Gonda et al., 1985). Briefly, the virus pellets were fixed with a 0.1M sodium cacodylate buffer containing 1.25% glutaraldehyde, pH 7.2, followed by a 1% osmium tetroxide in the same buffer. The fixed pellets were dehydrated in a series of graded ethanol solutions (35%, 50%, 75%, 95% and 100%) and propylene oxide. The pellets were infiltrated overnight in an epoxy resin (LX-112) and propylene oxide mixture, then embedded in epoxy resin to cured for 48 hours at 60C. Thin-sections (50 to 60 nm) of the pellet were cut, mounted on a naked copper grid, and double stained with uranyl acetate and lead citrate. The thin sections were stabilized by carbon evaporation in a vacuum evaporator, observed, and photographed with an Hitachi H-7000 electron microscope operated at 75kv.

The present invention has been described with respect to certain embodiments. The scope of the invention should not be limited to these described embodiments, but rather should be determined by reference to the following claims.

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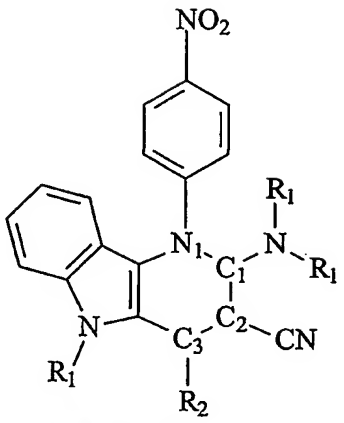
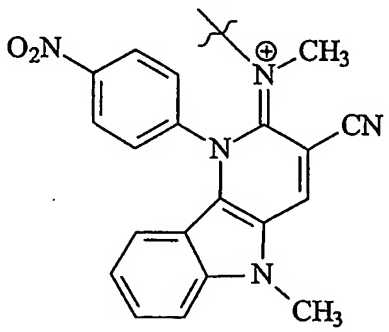
WE CLAIM:

1. A compound having Formula 1



Formula 1

where R_1 is independently selected from the group consisting of lower alkyl; N_1 is a nitrogen in a ring and is bonded to C_1 by either a single bond or a double bond; C_1 is a carbon atom in a ring, is bonded to N_1 by either a single bond or a double bond, and is bonded to C_2 by either a single bond or a double bond; C_2 is a carbon atom in a ring and is bonded to C_1 by either a single bond or a double bond, and is bonded by either a single bond or a double bond to C_3; and R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkyl amide, and Formula 2



Formula 2

2. The compound according to claim 1 where R_1 is methyl.

3. The compound according to claim 1 where N_1 is bonded to C_1 by a single bond.

4. The compound according to claim 1 where N_1 is bonded to C_1 by a double bond.

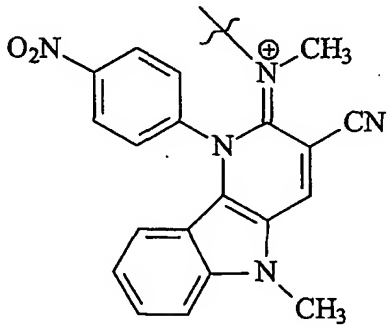
5. The compound according to claim 1 where C_1 is bonded to C_2 by a single bond.

6. The compound according to claim 1 where C_1 is bonded to C_2 by a double bond.

7. The compound according to claim 1 where R_2 is hydrogen.

8. The compound according to claim 1 where R_2 is lower alkyl amide.

9. The compound according to claim 1 where R_2 is

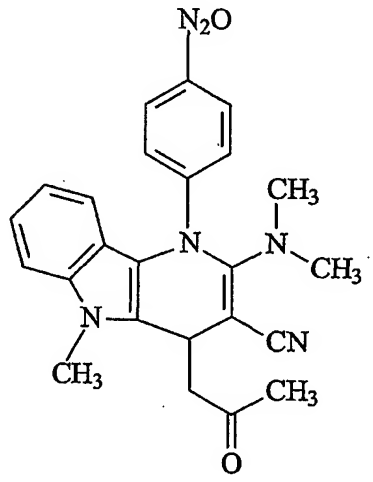


10. The compound according to claim 1 where R_1 is methyl, and N_1 is bonded to C_1 by a single bond.

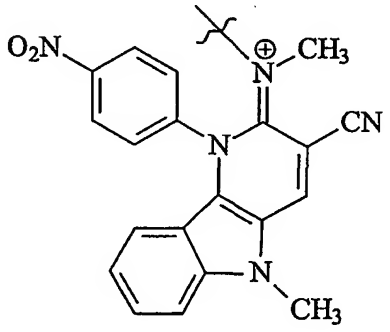
11. The compound according to claim 10 where C_1 is bonded to C_2 by a double bond.

12. The compound according to claim 11 where R_2 is lower alkyl amide.

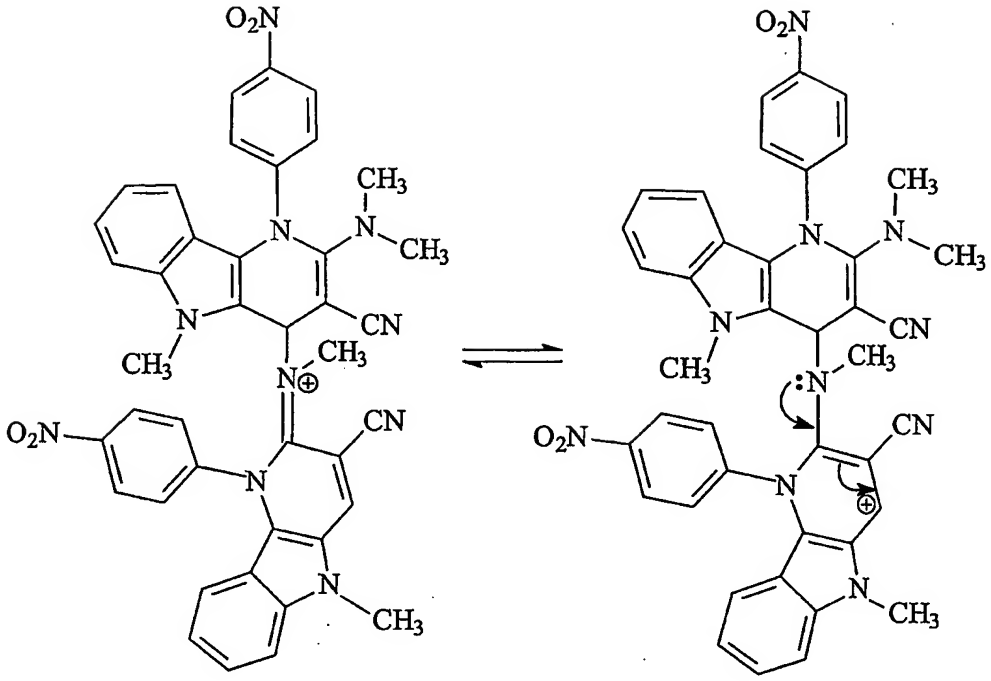
13. The compound according to claim 12 having formula



14. The compound according to claim 10 where R_2 is



15. The compound according to claim 11 having formula

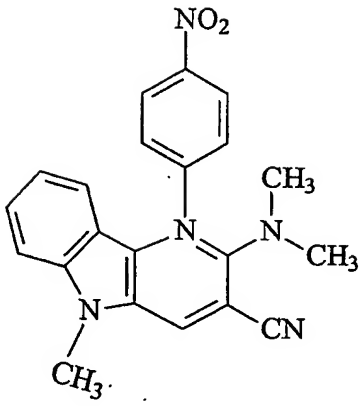


16. The compound according to claim 1 where R_1 is methyl, and N_1 is bonded to C_1 by a double bond.

17. The compound according to claim 16 where R_2 is hydrogen.

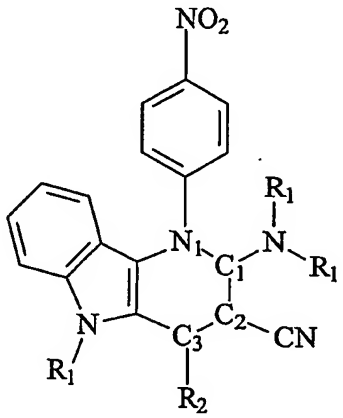
18. The compound according to claim 16 where C_1 is bonded to C_2 by a single bond.

19. The compound according to claim 18 having formula



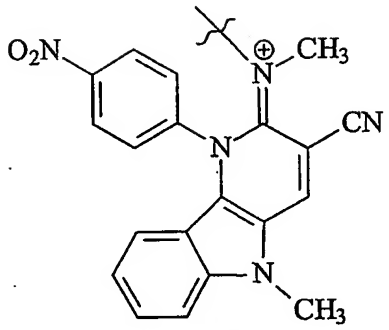
20. A method for treating a subject, comprising:

providing a compound having Formula 1



Formula 1

where R_1 is independently selected from the group consisting of lower alkyl; N_1 is a nitrogen in a ring and is bonded to C_1 by either a single bond or a double bond; C_1 is a carbon atom in a ring, is bonded to N_1 by either a single bond or a double bond, and is bonded to C_2 by either a single bond or a double bond; C_2 is a carbon atom in a ring and is bonded to C_1 by either a single bond or a double bond, and is bonded by either a single bond or a double bond to C_3; and R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkyl amide, and Formula 2



Formula 2

and

administering an effective amount of the compound to the subject.

21. The method according to claim 20 where R_1 is methyl.

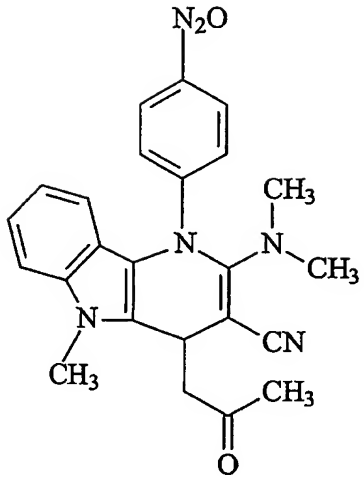
22. The method according to claim 20 where N_1 is bonded to C_1 by a single bond, and C_1 is bonded to C_2 by a double bond.

23. The method according to claim 20 where R_2 is lower alkyl amide.

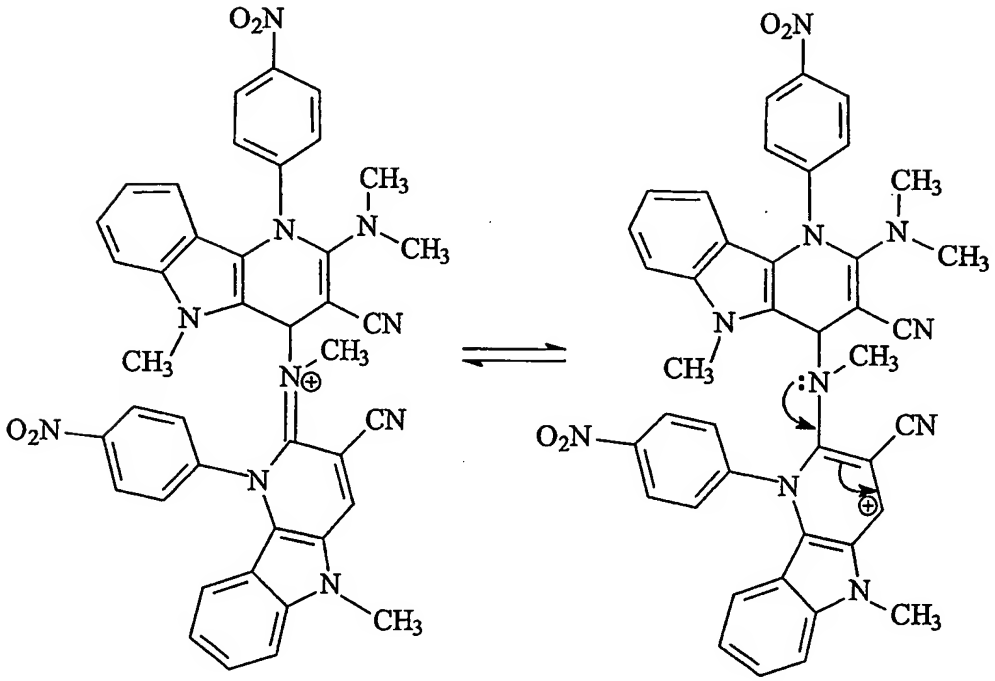
24. The method according to claim 21 where N_1 is bonded to C_1 by a single bond, and C_1 is bonded to C_2 by a double bond.

25. The method according to claim 24 where R_2 is lower alkyl amide.

26. The method according to claim 25 where the compound is



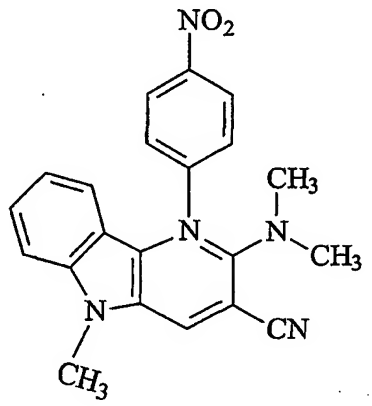
27. The method according to claim 25 where the compound is



28. The method according to claim 21 where N_1 is bonded to C_1 by a double bond.

29. The method according to claim 28 where C_1 is bonded to C_2 by a single bond.

30. The method according to claim 29 where the compound is



31. The method according to claim 20 where the subject is a mammal.

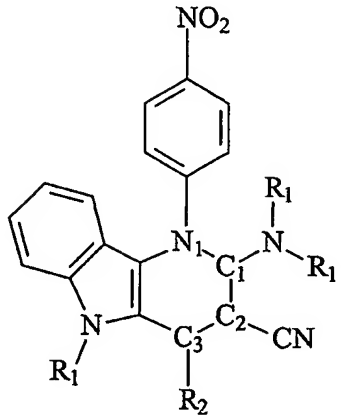
32. The method according to claim 20 where the subject is a human.

33. The method according to claim 20 where the effective amount is from about 0.1 mg/kg body weight per day, to about 200 mg/kg body weight per day, in single or divided doses.

34. The method according to claim 20 where the step of administering comprises administering compound or composition topically, orally, intramuscularly, intranasally, subcutaneously, intraperitoneally, or intravenously.

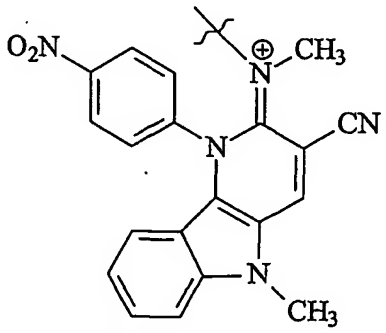
35. The method according to claim 20 where the compound is administered as a pharmaceutical composition.

36. A pharmaceutical composition comprising an effective amount of a compound having formula 1



Formula 1

where R_1 is independently selected from the group consisting of lower alkyl; N_1 is a nitrogen in a ring and is bonded to C_1 by either a single bond or a double bond; C_1 is a carbon atom in a ring, is bonded to N_1 by either a single bond or a double bond, and is bonded to C_2 by either a single bond or a double bond; C_2 is a carbon atom in a ring and is bonded to C_1 by either a single bond or a double bond, and is bonded by either a single bond or a double bond to C_3; and R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkyl amide, and Formula 2



Formula 2

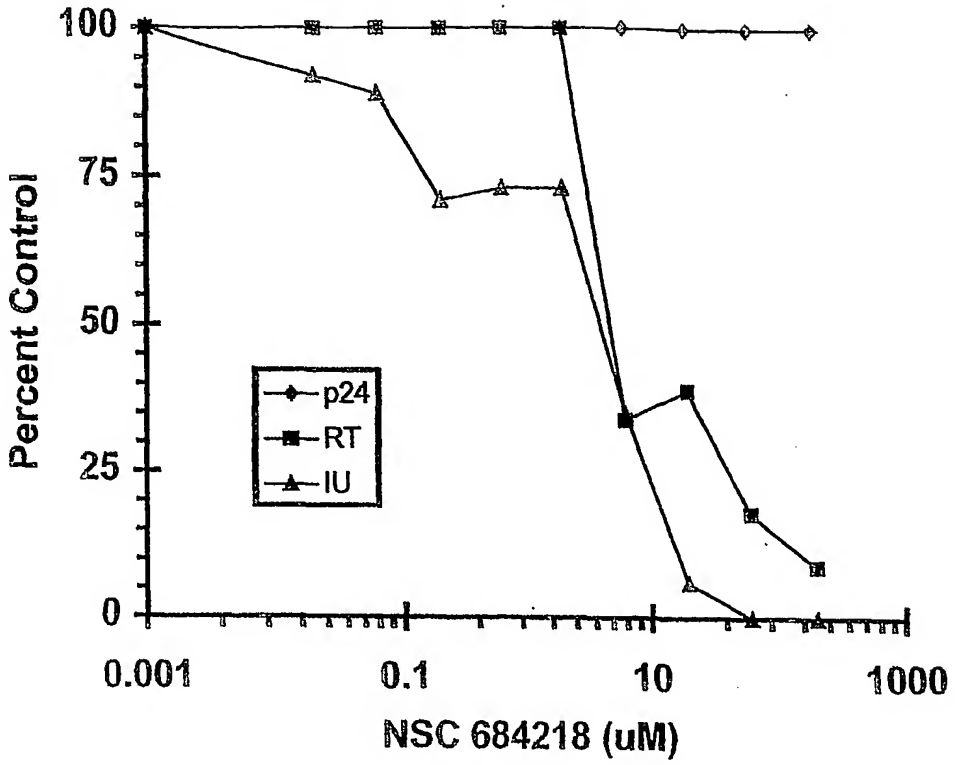
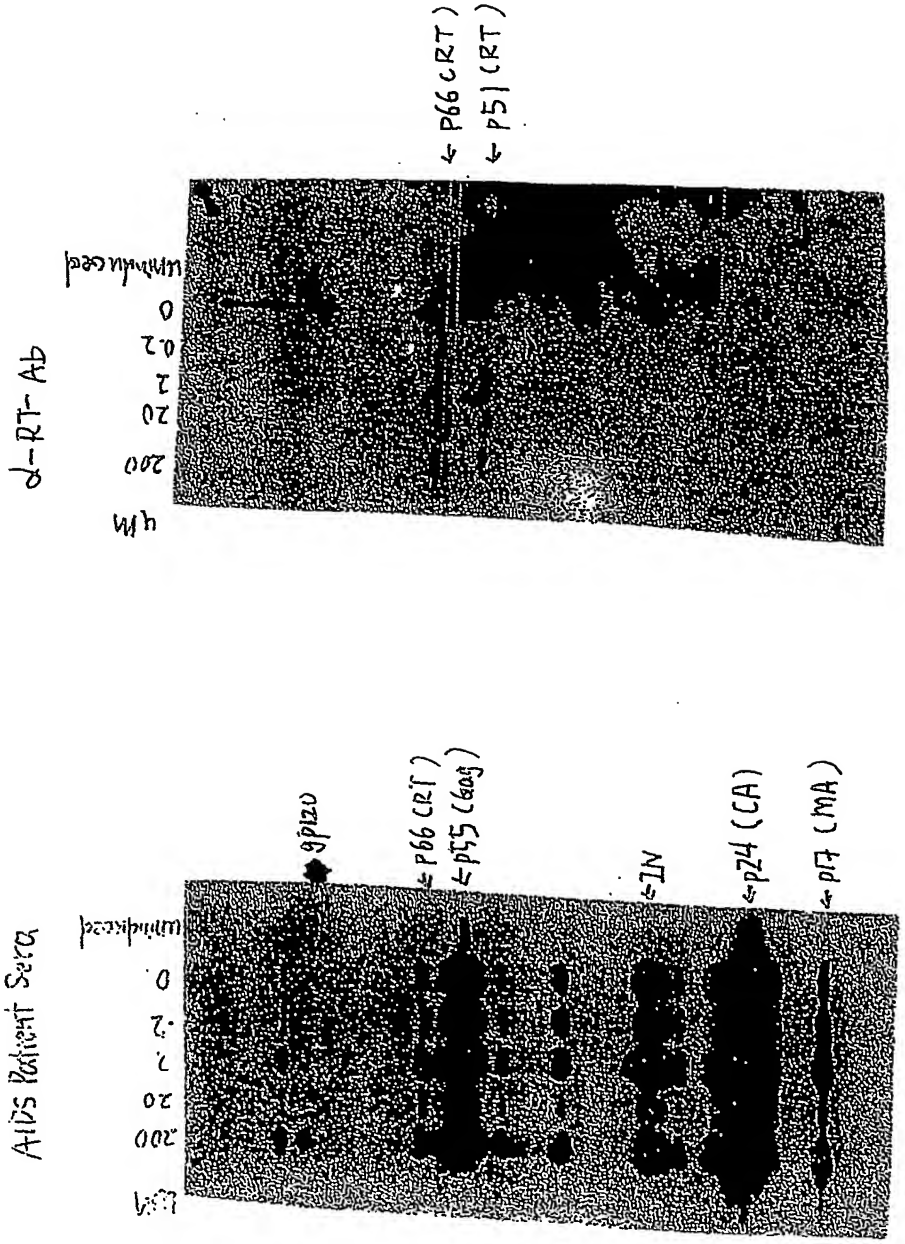

100
75
50
25
0
Percent Control
p24
RT
IU
0.001
0.1
10
1000
NSC 684218 (uM)

FIG. 1



Western blot analysis of virions produced from NSC 684218 treated chronic infected cells.

FIG. 2

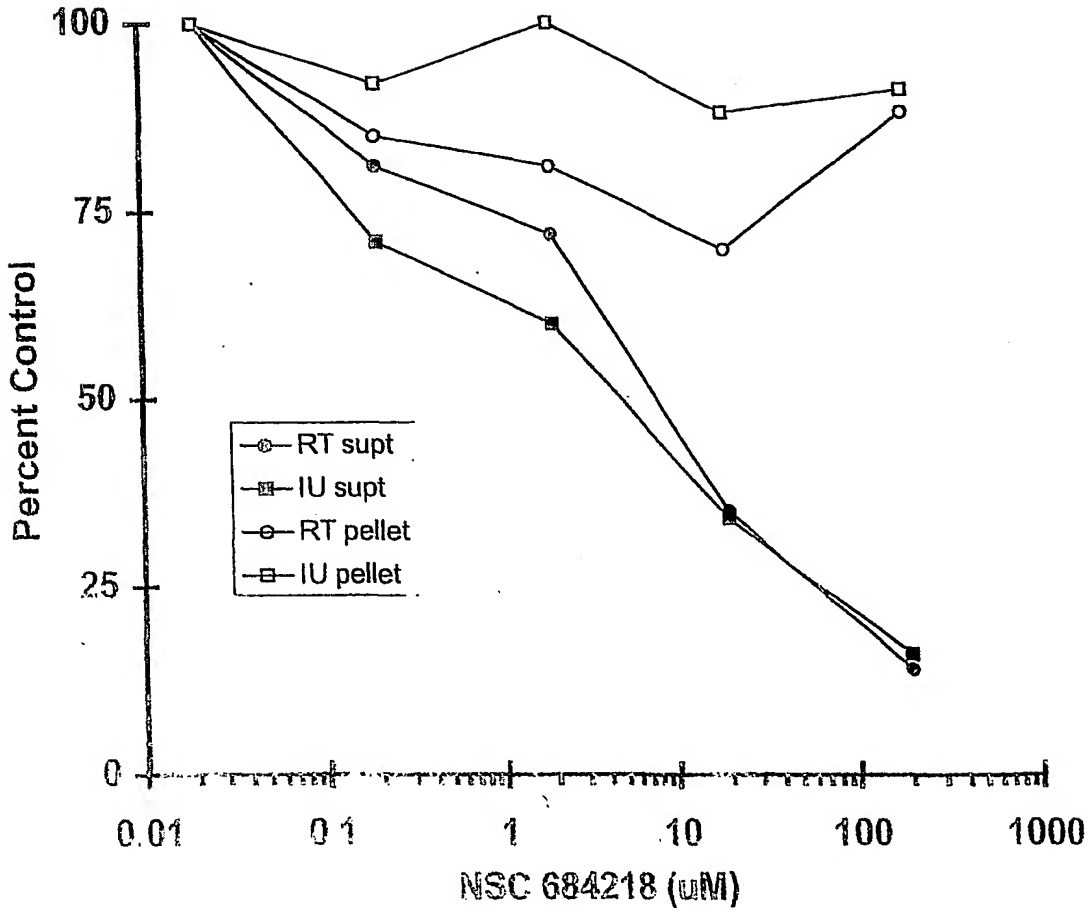


FIG. 3

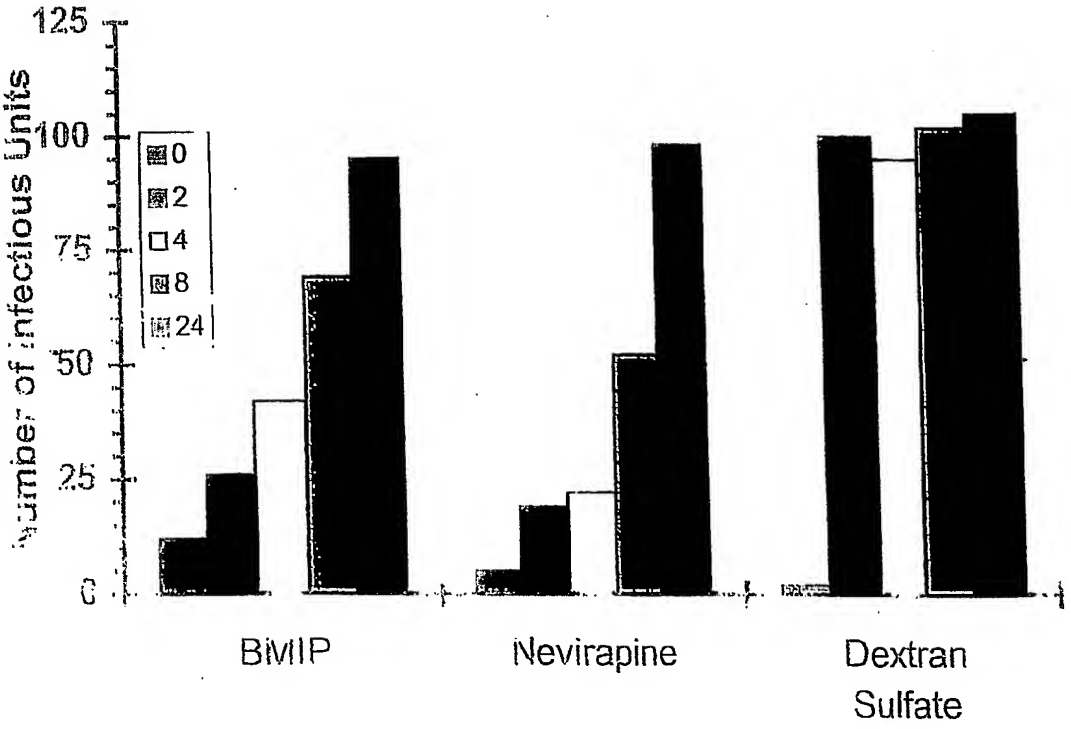


FIG. 4

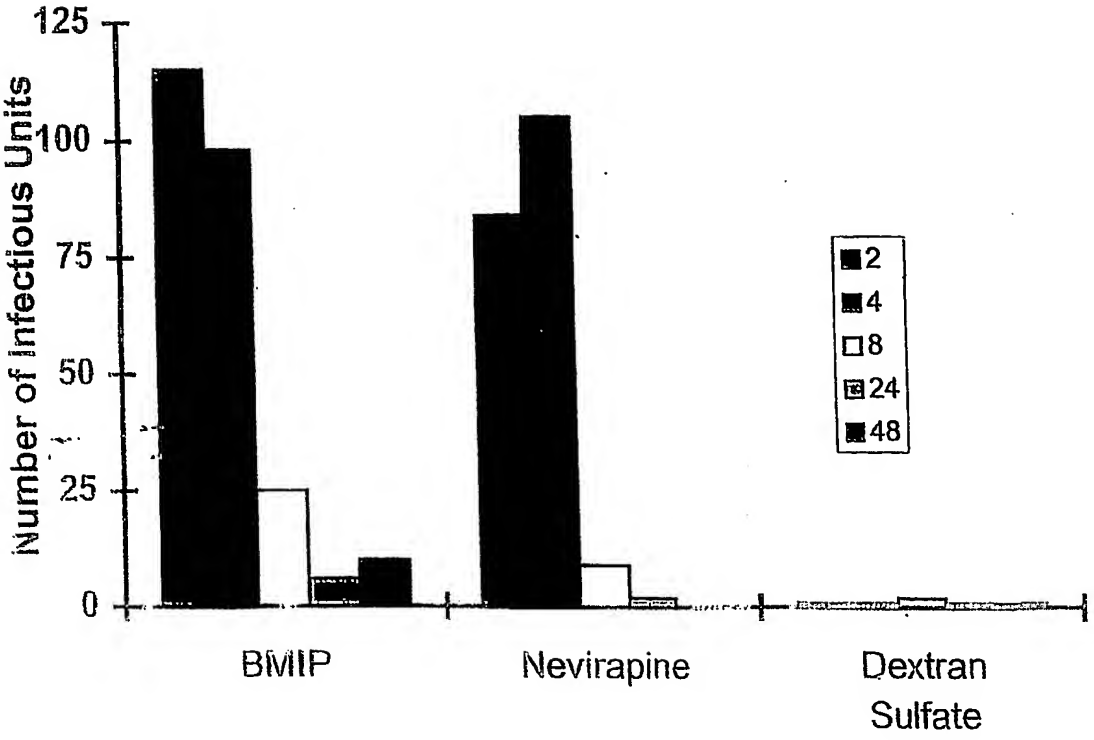


FIG. 5

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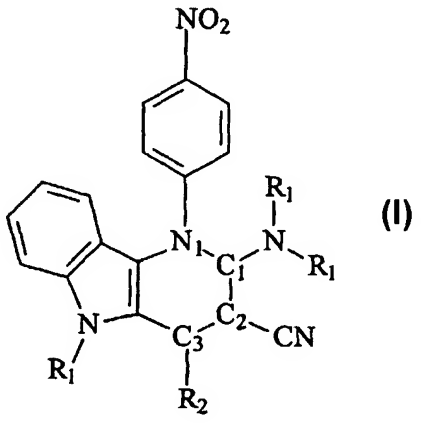
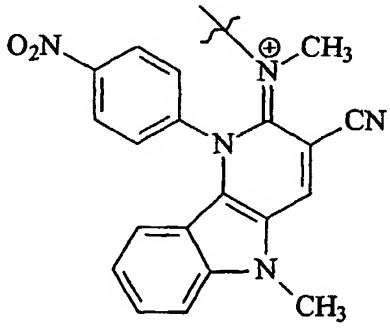
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,

[Continued on next page]

(54) Title: BENZOYLALKYLINDOLEPYRIDINIUM COMPOUNDS AND PHARMACEUTICAL COMPOSITIONS COMPRISING SUCH COMPOUNDS



(I)



(II)

(57) Abstract: The design, synthesis and antiviral activity of novel benzoylalkylindole-pyridiniums are disclosed. These compounds inhibit the reverse transcriptase enzymes of several retroviruses, including human immunodeficiency virus types 1 and 2, and simian immunodeficiency virus, and effectively inhibit viral replication thereby. Most of these compounds satisfy formula (1) where R_1 is independently selected from the group consisting of lower alkyl; N_1 is a nitrogen in a ring and is bonded to C_1 by either a single bond or a double bond; C_1 is a carbon atom in a ring, is bonded to N_1 by either a single bond or a double bond, and is bonded to C_2 by either a single bond or a double bond; C_2 is a carbon atom in a ring and is bonded to C_1 by either a single bond or a double bond, and is bonded by either a single bond or a double bond to C_3; and R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkyl amide, and formula (2). Compositions comprising effective amounts of such compounds are also described. These compounds and compositions can be used in a method for inhibiting the replication of retroviruses in a subject comprising administering an effective amount of the compound(s) or composition(s) comprising the compound, to a subject to inhibit retroviral replication.

WO 02/059123 A3

SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

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6 February 2003

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INTERNATIONAL SEARCH REPORT

[redacted] ional Application No
[redacted] /US 01/48311

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D471/04 C07D519/00 A61K31/437 A61P31/12
//(C07D471/04,221:00,209:00),(C07D519/00,471:00,471:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 62503 A (CNRS) 9 December 1999 (1999-12-09) page 9, line 29 -page 10, line 8; claims 1,4	1,36

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 October 2002	11/10/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Alfaro Faus, I

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/48311

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 20 - 35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

[redacted]rmation on patent family members

[redacted]onal Application No
[redacted]US 01/48311

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9962503 A	09-12-1999	EP 0966963 A1	29-12-1999
		AU 4368799 A	20-12-1999
		BR 9910810 A	13-02-2001
		WO 9962503 A2	09-12-1999
		EP 1079826 A2	07-03-2001
		HU 0102240 A2	28-11-2001
		JP 2002516851 T	11-06-2002
		NO 20006027 A	22-01-2001